TECHNICAL REPORT

The Biological and Toxin Weapons Convention

Implications of advances in science and technology

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This summary is designed as a record of issues that were discussed during the international workshop on advances in science and technology for the BWC, held 13 – 15 September 2015. The views expressed are those of the authors and do not necessarily represent the consensus opinions of workshop participants, the organizations that provided support for the project, or the 107 academies of science that form IAP nor mention of trade names, commercial practices, or organisations imply endorsement by the US or UK governments or other sponsors. Biosecure was commissioned to produce the background documents and initial drafts of the report documents.

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Introduction

The Biological and Toxin Weapons Convention (BWC) will hold its Eighth Review Conference late in 2016 which will consider 'new scientific and technological developments relevant to the Convention'. This review will draw upon work undertaken in a standing agenda item in the BWC's 2012 – 2015 work programme.

Each year, States Parties have reviewed developments in science and technology relevant to the BWC, focusing on advances that could have benefits for, or which could possibly be used in contravention to this international instrument. During the course of their work, States Parties supported by international organizations, scientific bodies, companies and individual experts, have reviewed developments in: enabling technologies; for dealing with disease; in the understanding of pathogenicity, virulence, toxicology, immunology and related issues; as well as production, dispersal and delivery technologies.

In September 2015, a Trends Symposium to review relevant developments in the science and technology was organised by the Royal Society, the US National Academies of Sciences and the IAP: Global Network of Science Academies, represented by the current Chair of the IAP Biosecurity Working Group, the Polish Academy of Sciences. The Symposium was attended by 72 participants from 30 countries.

A series of background documents[i] were commissioned to inform the discussions at the Trends Symposium, which were then reviewed in light of the presentations and discussions to produce this technical document and accompanying summary documents.[ii]

i. The background documents that this technical report draws on include the background information documents compiled by the BWC ISU, research on the BTWC process, presentations, statements and other contributions by States Parties, international organizations and Guests of the Meeting at meetings of the BWC from 2012 to 2014, and reports and outputs from international scientific and technical workshops and were commissioned from Biosecure.

ii. Available at iapbwg.pan.pl

Executive summary

The Trends Symposium and background documents noted many issues of relevance to the Review Conference and reached several overarching conclusions which are outlined below.

The Trends Symposium concluded that:

- Technological barriers to acquiring and using a biological weapon have been significantly eroded since the Seventh Review Conference, there had been no further novel developments that could enable activities inconsistent with the aims and objectives of the BWC.
- Likewise, there were no developments that would not be covered by the treaty or additional supplementary understandings, but
- The speed at which the life sciences and technology are advancing, and the rate of convergence of disciplines, is still accelerating. This increases the likelihood of such developments in the foreseeable future.
- Such potential problems, as well as any appropriate responses could be discussed prior to the 9th Review Conference.
- Biotechnology is increasingly important around the world as a manufacturing technology and has therefore become a potential target for biological weapons itself. It should be explored whether there are any risks not already captured by existing treaties and laws of weapons that cause damage to equipment supplies, or material associated with the bio-economy.
- There was an increased need for education and outreach to promote the aims and objectives of the BWC amongst the scientific community.

There were four themes that arose from the background papers and during the Trends Symposium around which this report is structured:

- Biosciences are developing at an unprecedented rate.
- The BWC should continue to monitor these developments.
- Global ability to detect and treat disease has been enhanced.
- Recent advances could facilitate the development of biological weapons.

Summaries of these sections are provided below and each is considered in detail in each chapter in the reminder of this report, including a synopsis of the research papers which lie behind the developments provided in the annotated references.

The biosciences are developing at an unprecedented rate

The rate and scale of progress in the life sciences and biotechnology continues to grow rapidly. There is an increasing diffusion of knowledge around the world and more interconnection between knowledge hubs, many of them ‘virtual’. Laboratories operate in more diverse geographic locations and across different sectors of our societies.

The character of life sciences and biotechnology is evolving with greater focus on rational design, biological engineering, and more flexible production. Moving from ‘concept’ to ‘application’ is becoming ever simpler, unlocking further potential for progress. The adoption of engineering paradigms has contributed to progress.

All these factors have both positive and negative implications for the BWC.

The BWC should continue to monitor these developments.
For this reason, the BWC should continue to ensure that such developments are not used in prohibited activities, whilst facilitating their use for peaceful purposes. It could achieve this by devising an effective, on-going, and suitably resourced mechanism to:

- Develop specific questions that can be answered through an on-going review of developments in science and technology;
- Identify current scientific and technical capabilities applicable to these questions;
- Consider the implications of those developments in the context of the BWC; and
- Formulating informed decisions about any further actions that may be required.

The conference observed that the development of a technique for systematically assessing the risk of new scientific developments to the BWC would greatly assist in this process.

The global ability to detect and treat disease has been enhanced.
The meeting noted that one positive outcome of developments since the Seventh Review Conference is that our collective capacity to combat disease has markedly improved, regardless of whether the outbreak is naturally occurring or the result of a malevolent act. Whilst it would require remaining logistic, economic and technical barriers to be surmounted, the conference noted that it should now be possible to assemble patchwork capabilities into a diffuse but integrated system for countering global or local outbreaks.

Such a system could scale from local needs through to international responses. A structure that enabled data such as pathogen sequences to be shared more effectively and efficiently would facilitate a rapid an effective response. As expertise and 'know-how' matures, opportunities for technological leapfrogging appear, as was the case with mobile communication systems. Developing countries can then access opportunities and capabilities in the field that match, if not surpass, those found in developed countries.

Increasing 'digitization' of pathogen data enables the identification and characterisation by centralised facilities of the infectious agent facilitating development of countermeasures and the use of 'distributed diagnostics' at the site of the outbreak. This concept has already been partially implemented in both developed and developing countries. The conference noted a pressing need for more comprehensive sets of baseline and reference pathogen data. 'Microbial forensics' can be used to help establish attribution if a malevolent deployment is suspected.

Vaccines and drugs can now be developed more rapidly than ever before. Lead times can be reduced through rapid detection and characterisation of outbreaks. The design, testing and optimisation of vaccines and drugs have been streamlined using better computational technologies, modelling tools and platform technologies although provision of bioinformatics capabilities remain challenging.

Outsourcing of key production steps has reduced the need for dedicated vaccine production infrastructure. It is increasingly simpler, faster and cheaper to industrialize production processes. Single-use equipment and modular production technologies shorten turn-around times. A more distributed production base in industry reduces the distance a product has to travel to its point of use. However, regulatory and liability issues associated with diagnostics, drugs and vaccines in health emergencies continue to limit potential for progress and this is an issue that should be addressed.

Recent advances have also facilitated the development of biological weapons.
The scientific advances reported at the conference could also facilitate almost every step of a biological weapons programme and technological barriers to acquiring and using a biological weapon have been conspicuously eroded since the Seventh Review Conference.

Both novel and traditional approaches continue to offer opportunities for acquiring an agent from nature. The sometimes-formidable challenges associated with the synthesis of existing agents and the development of novel agents have been overcome by using gene transfer and other biosynthetic engineering approaches.

Modification of biological agents enables them to be more easily optimised for use in a biological weapon. Developments in scale-up and production technologies have changed production signatures. Less space and time are needed, narrowing windows for interdiction.

Although these trends diminish the need for stockpiling, the proliferation of such capabilities, such as freeze-drying capacity, has actually reduced the space required to store biological weapons. It is also now easier to deliver a biological agent given advances in areas such as nanoparticles and sophisticated modelling of dispersal patterns using the techniques of aerobiology.

Many of these advances are at the leading edge of current capabilities. They are expensive and complicated to acquire and to deploy successfully. Making use of them for prohibited purposes would probably currently require the resources of a state but this situation may change in the future, reinforcing the need for on-going efforts to review relevant developments in science and technology.

1

Scope of the BWC

The upcoming Review Conference will consider whether there had been any novel developments since the Seventh Review Conference that could either enable activities inconsistent with the aims and objectives of the treaty, or which would not be covered by the BWC or additional understandings reached at subsequent review conferences. No concrete examples of such developments were identified either through the literature reviews or in workshop discussions.

However, a number of potential future scenarios were identified that do give cause for concern. Situations where, for example, the mechanisms of action of weapons are not clearly 'chemical' or 'biological', where components are significantly different from existing biological systems, or where inorganic materials mimic biological function, thereby having a biological effect.

It was easy to envisage how progress in areas such as the convergence of the sciences, nanotechnology and therapeutic design, might lead to such concerning developments. States Parties could proactively consider the implications of these scenarios before they can be realised, thereby providing a window of opportunity to develop appropriate responses and actions.

Given the increasing pace of progress and the additional possibility of sudden 'non-linear' advances in research fields, it is difficult to estimate when such potential scenarios might become relevant to the BWC. Further consideration of progress in this area may be needed prior to the 9th Review Conference and this reinforces the importance of a flexible process for on-going reviews (see Section 2).

Biotechnology is an increasingly important global manufacturing technology. Degrading manufacturing infrastructure and capability has long been a tool of war, insurgency and armed conflict. The 'bio-economy' itself is therefore a potential target for biological weapons. The BWC currently prohibits weapons that cause harm to humans, animals and plantsand it should be explored wether they are any risks, not already captured by existing treaties and laws of weapons that cause damage to equipment supplies, or material associeted with bio-econommy.

The conference also noted an increased need for education and outreach to promote the aims and objectives of the BWC amongst the scientific community as well as the provision of responsible mentorship for early-stage researchers. There are already some examples of excellence in this area (see Box 1) but more effort is needed by States Parties and 'best practices' disseminated.

Box 1: Synbio LEAP – An example of excellence in socially responsible scientific mentorship

The Leadership Excellence Accelerator Program (LEAP) provides Fellows with mentorship, practical skills and a sustaining network to help them guide a socially responsible future for synthetic biology. It is an incubator for emerging leaders across disciplines and sectors to develop new strategies for biotechnology in the public interest.

LEAP is an intensive year-long non-residential Fellowship program. Each round, twenty fellows participate in two in-residence workshops during which Fellows work together to develop strategies to address their top challenges for the practice of synthetic biology, under the guidance of world-class experts across disciplines and sectors.

Image
Participants at a LEAP meeting at the Wilson Center, Washington DC.
Photo courtesy of David Sun Kong Photography.

2

Monitoring developments in science and technology

It is clearly desirable for the BWC to ensure that new knowledge and techniques do not facilitate breaches of the treaty, whilst expediting their peaceful applications. This requires an understanding of how developments in the life sciences and biotechnology might impact the treaty.

There is therefore a pressing need for effective, on-going, and suitably resourced arrangements to:

- **Formulate** specific questions that can be answered by reviewing developments in science and technology;
- **Identify** current scientific and technical capabilities pertinent to these questions;
- **Consider** the implications of those developments in the context of the BWC; and
- **Take** decisions or actions necessitated by those developments.

Models used by other international bodies, including disarmament forums, environmental or health treaties could be adapted for this purpose. The advantages and disadvantages of these different formats should be explored in the context of the BWC's remit.

Whatever processes is eventually implemented, it should be:

- **Flexible:** able to adjust to changing needs and priorities;
- I**nclusive:** able to take advantage of expertise, wherever it is to be found;
- **Agile:** able to adjust quickly to seize all available opportunities;
- **Responsive:** able to actually change when needed;
- **Able to foster greater engagement:** actively encouraging and enabling contributions from the widest possible set of stakeholders; and
- **Transparent:** ensuring the widest possible group of stakeholders benefit from its work.

The Trends Symposium observed that the development of a technique for systematically assessing the risk of new scientific developments would be of enormous help in assessing the threat of future trends. Such a process might also usefully consider criteria for identifying relevant or significant developments.

3

The global ability to deal with disease

Since the Seventh Review Conference, developments in the understanding of disease have increased capacity to deal with disease, regardless of whether the outbreak is naturally occurring or the result of a malevolent act.

The following sections consider each of the following factors which have contributed to this:

- Understanding disease
- Detecting technology
- Diagnosis and surveillance
- Drugs (including vaccines and therapeutics)
- Responses

3.1 Understanding disease

Improved understanding of disease mechanisms has increased our capacity to detect, respond to and mitigate outbreaks, regardless of their source. Such developments strengthen capacities relevant to Articles VII and X of the BWC.

Particularly noteworthy is our enhanced understanding of transmissibility and host range; pathogenicity and virulence; toxins; unusual disease agents (including prions and fungi); immunology and host-pathogen interactions; the role of the microbiome; and the significance of 'biofilms' for pathogen persistence.

3.1.1 Transmissibility and host range

Since the Seventh Review Conference there have been incremental increases in our understanding of transmissibility and host range. This includes structural and genetic analysis, production or isolation of pathogens with increased transmissibility or host range and improved tools to investigate these.

Transmissibility

Examples of genetic sequences or mutations that have been identified include:

- mutations in H5N1,[1, 2, 3, 4, 5] H7N1 influenza virus[6, 7] and H1N1 influenza viruses[8, 9] which confers aerosol transmission in mammals;
- mutations in HA region of H5N1 influenza virus that improves affinity for humanlike airway receptors;[5]
- a mutation in H7N9 influenza that confers an ability to bind to both human and avian receptors;[10]
- the sequence of a H7N9 influenza virus adapted for efficient growth in human lung tissue;[11]
- mutations in H7N9 influenza virus conferring antiviral resistance with no impact on transmissibility;[12] and
- mutations associated with host range.[13]

Pathogens with increased transmissibility have been produced or isolated:

- a H5N11 and H1N15 recombinant influenza viruses capable of aerosol transmission in ferrets;[1, 5]
- the use of site-directed mutagenesis and serial passaging in ferrets of the H5N1 and H7N1 influenza virus to confer airborne transmission;[2, 6]
- a H7N9 influenza virus adapted for efficient growth in human lung tissue;[11]
- a recombinant human and bat influenza virus;[7] and
- the use of serial passaging of an engineered re-assorted H1N1 influenza virus in pigs.[9]

Host range

Identification of key structural elements that affect host range, including how the structure of HA regions affect H5 influenza viruses ability to bind to avian and human receptors;[14, 15] and the H7N9 influenza viruses[15, 10]

Improved understanding of the interconnections between pathogenicity, transmissibility and drug resistance identification of mutations that can confer additional functional characteristics without degrading others.[12]

Pathogens with an altered host range have been produced, including:

- enteroviruses type 71;[4]
- H1N1 influenza virus;[4]
- H5N1 influenza virus;[4] and
- Dengue fever virus.[4]

Tools

More sophisticated tools for identifying factors associated with transmissibility and host range and integrating them into agents have been developed, such as:

- tools to study genetic evolution of viruses and search for mutations known to confer altered transmissibility;[3]
- synthetic biology approaches;[16]
- approaches for characterizing host shifts for rabies viruses in bats[17] and Ebola virus between pigs and non-human primates; and
- model systems that recapitulate *in vivo* viral life cycles.[18]

3.1.2 Pathogenicity and virulence

Since the Seventh Review Conference there have been incremental increases in our understanding of what causes pathogenicity and virulence. This includes structural and genetic analysis, investigation of other factors which effect virulence (infection location, acclimatisation) and improved tools to investigate these.

Genetic and structural analysis

Identification and characterization of genetic components conferring pathogenicity in the following organisms, such as:

- a particularly lethal Escherichia coi (O104:H4);[19]
- SARS coronavirus;[20]
- HIV;[20]
- enteroviruses type 71;[21]
- influenza A H1N1 virus;[21]
- H5N1 influenza viruses; [21]
- Dengue fever virus; [21]
- methicillin-resistant *Staphylococcus aureus*;[22]
- *Mycobacterium tuberculosis*;[22]
- an unculturable bat virus;[7]
- H7N9 influenza virus,[23, 24] further work on indicated:[25]
- *Bacillus anthracis*;[26] and
- wheat yellow rust.[27]

Identification and characterization of transcription signatures connected to pathogenicity e.g. in H5N1 and H1N1 influenza viruses.[28]

Identification of key structural elements that affect pathogenicity including:

- intercellular nanotubes used to exchange plasmids, including those containing pathogenicity factors;[29]
- mapping and characterisation of infection-specific structures in rice blast;[30]
- changes in the HA and NA regions of human and bat influenza viruses;[7]
- type III secretion systems used by pathogens, such as *Salmonella typhimurium*, to manipulate host cells;[31]
- how hydrophobic structures in the core of viral capsids can be crucial for infectivity;[32] and
- partial characterization of the virus-host structural components in a particularly virulent strain of H7N9 influenza virus.[33]

Other factors

Improved insights into other factors that impact pathogenicity, such as physical location in the host and the need to adapt to a new temperature. Some examples include:

- bacteraemia in inhalational anthrax infections – a novel model of which suggests that higher levels of mortality witnessed in inhalational anthrax is due to bacteria present in the lymph nodes, rather than presence in the lungs;[34] and
- a requirement for acclimatization to the host's temperature for the production of virulence factors in *Yersinia pestis* infections.[35]

Interconnections between pathogenicity, transmissibility and drug resistance e.g. mutations that can confer additional functional characteristics without degrading others.[12]

Tools

More sophisticated tools for identifying factors associated with pathogenicity and integrating them into agents have been developed, including:

- the use of sequencing technologies to identify new agents and pathogenicity factors;[36, 37]
- improved understanding of how pathogenicity factors spread in the wild, such as' horizontal gene transfer;[38]
- computational approaches and software tools for the identification of pathogenicity islands and virulence factors;[22, 39, 40]
- synthetic biology approaches;[16, 20, 41]
- bioinformatics tools for manipulating pathogenicity data;[41]
- systems biology and understanding the impacts of systems modulation on disease;[41]
- improved mechanisms for combining sequence data and structural analysis for detecting virulence factors;[37]
- whole genome 'directed evolution';[16]
- methodological approaches for testing the clinical and public health significance of microbe-disease associations;[42]
- approaches for predicting the virulence of a pathogen from its sequence information;[22]
- the recreation of a centralised repository for pathogenicity islands and resistance islands;[43] and
- model systems that recapitulate *in vivo* viral life cycles.[18]

Increased virulence

Pathogens with increased virulence have been produced:

- the use of serial passaging of Schmallenberg Virus in a mouse model;[44]
- the use of serial passaging of H7N1 in ferrets;[6]
- recombination of influenza viruses to create a novel virus that is functionally similar to the causative agent of the 1918 pandemic but with increased pathogenicity;[8]
- the use of serial passaging of an engineered reassortant swine influenza virus in pigs;[9] and
- the use of serial passaging of Ebola virus in guinea pigs.[13]

There has been progress in identifying ways in which pathogens cause damage by manipulating their host, for example, modulating the damage caused by cytokine storms[45, 46] or inflammatory responses.[46, 47]

3.1.3 Toxins

Since the Seventh Review Conference there have been incremental increases in our understanding of the production and action of toxins. This includes structural and genetic analysis, characterisation of the mechanisms of action and novel toxins and drugs to neutralise toxins, detection procedures and improved tools to investigate these. There has been increased interest in toxins for applications in medical treatment, life sciences research, pharmaceuticals, and agriculture.[48]

Genetic and structural analysis

Identification and characterization of genetic components associated with toxins, including:

- genome-wide RNAi screens identifying genes required for intoxication from Ricin and Pseudomonas exotoxin;[49]
- 743 mutations in 12 human genes important for intoxication by four different cytolethal distending toxins;[50]
- human genetic variation that determines sensitivity to the anthrax toxin;[51]
- publication of sequence and transcription data for king cobra venom;[52] and
- genetic elements associated with Botulinum neurotoxins.[53]

Improved understanding of interactions between genetic elements also plays an important role in the production and action of toxins, for example, gene interaction mapping of Ricin toxin.[54]

Characterisation of mechanism of action

Characterization of the mechanisms of action associated with toxins:

- increased understandings of the cellular mode of action of Ricin toxin;[55]
- characterization of the mechanism by which Botulinum neurotoxins are taken up at the pre-synaptic nerve terminal;[56]
- characterization of the metabolic pathway that produces Saxitoxin;[57]
- modulation of binding immunoglobulin protein to increase cytotoxicity;[58]

- characterization of the mechanism of action of the monoclonal antibody which neutralises Abrin;[59]
- characterization of the mechanism of action of monoclonal antibodies targeting both Ricin A and B chains;[60]
- characterization of a novel Botulinum neurotoxin;[61]
- characterization of the absorption, distribution and pathological injury in mice due to Ricin poisoning via the alimentary pathway;[62]
- insights into the general circulation, neuronal binding, membrane translocation and neuroparalysis Botulinum neurotoxin administration;[53] and
- details of structural interactions between Botulinum toxin and host cells.[63]

Tools

More sophisticated tools for researching and manipulating toxins have been developed, such as:

- synthetic biology approaches;[41, 48]
- bioinformatics tools for manipulating environmental stability data;[41]
- systems biology and understanding the impact of biological systems modulation on disease;[41]
- metabolic pathway engineering;[64] and
- computational design tools leveraging advances in understanding in structural-functional relationships.[65]

Novel toxins and drugs

Novel toxins, or those with altered or enhanced characteristics have been identified or produced, including:

- an anthrax toxin with altered receptor specificity;[66]
- a novel form of Botulinum toxin;[67]
- a robust high-content screen developed to discover novel compounds that stabilize intracellular Ricin and limit Ricin intoxication;[68] and
- a recombinant Botulinum type A – Tetanus toxin.[69]

Novel drugs, drug targets and drugs that neutralise the effect of toxins have also been developed:

- a compound to block the uptake of Botulinum neurotoxins at the pre-synaptic nerve terminal;[56]
- a monoclonal antibody capable of neutralizing the Ricin toxin;[70]
- sub-domains of Ricin's B subunit now targeted by toxin neutralizing and non-neutralizing monoclonal antibodies;[71]
- competitive active-site inhibitors of Ricin Toxin A;[72]
- comparative analysis of monoclonal antibodies target the A and B chains of Ricin;[73]
- demonstration that fragments of monoclonal antibodies function as effectively as full length compounds in neutralizing Ricin in a cell based assay;[74]
- monomeric single-chain camelid antibodies capable of neutralizing Ricin in vitro;[75]
- an engineered heterodimeric camelid antibody capable of neutralizing Ricin *in vivo*;[75] and
- the use of intact IgG from goat anti-ricin hyperimmune sera to provide immediate protection following Ricin exposure and to confer an active immunity against Ricin that subsequently results in long term protection.[76, 77]

Detection

Shortcomings at the national and laboratory level were identified in maintaining and ensuring standards, methodologies and reference materials for detecting toxins.[78]

3.1.4 Unusual disease agents (including prions and fungi)

Since the Seventh Review Conference there have been incremental increases in our understanding the role of disease causing agents other than bacteria and viruses.

Fungi

High profile reviews focusing on human, animal and plant diseases caused by fungi, including significant historical epidemics.[79]

Plasmodia

Continued interest in diseases caused by plasmodia, including the identification of genetic elements associated with malaria.[80]

Prions

There has also been progress in understanding prions, how they cause disease and potential therapeutic interventions, including:

- the identification of genetic elements that when overexpressed significantly reduce the incubation time of prion diseases;[81]
- the role that the mis-folded proteins (usually associated with a diseased state) play in healthy neurological development;[82] and
- the development of a novel screening process to identify therapeutics.[83]

3.1.5 Immunology and host-pathogen interactions

Our understanding of host-pathogen interactions has also progressed since the Seventh Review Conference. These include modulating immune response to pathogens, characterisation of how pathogens evade the immune system and better understanding of the causes of side-effects produced following treatments with small molecule anti-infective drugs.

Expanding range of pathogens

Host-pathogen relationships are also being studied in an expanding range of pathogens:

- Enterobacteriacea;[38]
- influenza viruses;[84, 85, 86, 87]
- *Yersinia pestis*;[35, 45, 47, 87, 88, 89]
- Schmallenberg Virus;[44]
- *Mycobacterium tuberculosis*;[90]
- *flaviviruses*[91]; Dengue virus;[87]
- SARs coronavirus;[87]
- *Salmonella enterica*;[87]
- *Brucella* sp.;[87]
- *E.coli*;[87]
- West Nile virus;[87]
- *Mycoplasma* sp.;[87]
- varicella zoster virus;[87]
- vaccinia virus;[87] and
- *Staphylococcus aureus*.[92]

Modulating immune response

Developments in understanding host-pathogen relationships enabling the modulation of immune responses in the host:

- reducing or removing cytokine storms[93] and their impact on clinical outcomes;[94]
- the inflammatory mechanisms of gut bacteria, including the horizontal gene transfer of key factors;[38]
- novel functional characterization of B cells and autoantigens;[95]
- the impact of human genetic variation on sensitivity to anthrax toxin;[96]
- the identification of a novel neutrophil-based immune response to viruses;[97]
- the use of non-structural proteins to interfere with a host's epigenome and make cells more susceptible to viruses;[84]
- modulation of host proteins which inhibit viral replication;[85]
- infection kinetics and the impact of the physical location of pathogens in a host on the speed of infections;[88]
- mechanisms through which pathogens can interpret extracellular stimuli;[98]
- plant immune receptors and their interaction with plant pathogens and virulence proteins;[99]
- the use of natural (e.g. polysaccharide) or manmade (e.g. polyelectrolyte based nano-thin polymer) coatings to isolate cells from the host's immune system;[87, 100]
- gene silencing in a virus to enable a dormant state in the host's neuron's, dramatically increasing the persistence and re-emergence of infections;[22]
- degradation of the functional capacity of host macrophages, enabling pathogens to survive inside them; [87]
- characterization and comparison if the host immune response to H7N7, H5N1, and H1N1 influenza viruses;[28] and
- a novel bacterial mediator of pulmonary inflammation in pneumonic plague.[101]

Pathogen evasion of the immune system
There have been significant developments in understanding the mechanisms through which pathogens evade the host's immune system:

- proteins used by pathogens to suppress immunity in plants;[102]
- mechanisms for negating innate immunity;[22, 35, 47, 86, 87, 89, 91]
- mechanism for negating vaccine-acquired adaptive immunity;[22, 89]
- modulation of the production of viral proteins used to block interferon production in the host;[44]
- a viral mechanism using RNA motifs to avoid a host immune response;[103]
- chemical modification of the pathogen's genetic material to void detection;[87]
- the production of proteins by a pathogen to interfere with the host's ability to trigger an immune response,[87] including type III secretion processes; antigenic variation;[87]
- the use of TAM receptors to inhibit innate immune response;[87]
- interference with receptor proteins on host immune cells;[87] and
- broadly-reactive antibody-binding proteins used to block antibody-antigen binding.[87]

Drug side-effects
There have been new insights into hazardous side effects caused by drugs, for example, better characterization of the mechanisms responsible for hypersensitivities to small molecule drugs used for the treatment of infection.[104]

3.1.6 Improved tools to characterise disease

In general, there has been continued progress in enabling technologies offering 'many benefits in faster, cheaper, and easier application of biological science and technology for both public health and security purposes, increased capacity and better understanding of disease and healthcare technologies by more people in more locations throughout the world'.[105]

In particular there have been advances in sequencing technologies, including linking sequencing and functional data, working with large datasets, complex disease models, structural analysis, neurobiological techniques, tracking infection in the host, agent identification and drug development.

Tools developed since the Seventh Review Conference can characterise disease arising from infection with a range of agents of relevance to the BWC, including *Yersinia pestis*,[88] HIV,[32] foot-and-mouth disease virus,[106, 107] and toxins, such as cytolethal distending toxins,[50] and Ricin.[54]

Sequencing
Advances in sequencing technology now provide:

- improved identification of agents;[36, 42, 108]
- enhanced epidemiology and near real-time surveillance;[36]
- better tools for managing disease outbreaks;[36]
- techniques for establishing the limits of the use of genetic elements to predict disease course;[109]
- the use of genetic interaction mapping to identify functional relationships between genes;[54] and
- the use of transcriptomics to provide insights into plant pathogens that would otherwise be difficult to study.[27]

Developing superior tools to link sequence and functional data:

- connecting deep sequencing data with global gene disruption to allocate genes to phenotypes;[50]
- using genome wide association studies with non-targeted metabolomics to establish linkages with metabolic phenotypes;[110] and
- the re-creation of databases to house details of pathogenicity islands and resistance islands.[43]

Datasets and models
Improved tools and technologies to address the significant challenge of manipulating and analysing the massive amounts of data being generated by automated, high throughput technologies, for example, distributed computing technologies.[111]

Developing models to describe complex biological systems and to use them to further understandings of disease, for example a model of the capsid of foot-and-mouth disease virus was used to explain physical characteristics of the virus[106] as well as biological functions.[107]

Structural analysis

Improved techniques for structural analysis:

- techniques for stabilising receptors allowing previously uncharacterized structures to be determined using x-ray crystallography;[112]
- combined use of cryo-electron microscopy and cryo-electron tomography to identify the structure and molecular dynamics of a mature viral capsid;[32] and
- room-temperature macromolecular crystallography.[113]

Neurobiological techniques

There have been a broad range of advances in tools for studying neurobiology, including:

- in optogenetics where light is used to switch specific neurons, or classes of neurons on and off in a living host – helping to determine the role of those neurons in certain physiological states;[114, 115, 116]
- techniques for making biological material transparent to light, allowing neurological reactions to be studied in real time in a living host;[117] stimulating the neurons identified;[118] and for elucidating form-function relationships;[116, 119] and
- translation of such approaches and techniques for work in non-human primates.[120]

Nanotechnology

The continued integration of nanotechnology in relevant tools has led to new capacities for example to measure the cellular secretion of growth factors, cytokines, and other signalling molecules into extracellular spaces.[121]

Tracking infection in living hosts

Progress has been reported in being able to track infections in living hosts:

- the use of bioluminescence to study the kinetics of infection and the physical movement of a pathogen during different stages of disease course;[88]
- fabrication, characterization, and testing of a polymer 'microprojection array' for the direct and selective capture of circulating biomarkers from the skin.[122]

Agent identification

Sequencing technologies,[36] metagenomics technologies,[108] and mass spectrometry[123] are platforms that have all been used to identify agents more rapidly and reliably.

Drug development

Better tools for drug development are available, including:

- the use of 3D printed cells and tissues for the pre-clinical modelling of drug impacts and side effects;[124]
- the identification of a protein useful for purifying, immobilizing, and detecting all types of human and nonhuman immunoglobulin G antibodies;[125]
- biodegradable silicon nanoneedles to deliver nucleic acids into cells;[126] and
- further humanized forms of mouse antibodies.[127]

3.1.7 The role of the microbiome in disease

There has been an improved understanding of how communities of commensal microbes contribute to healthy and diseased states:[128]

- The Human Microbiome Project Consortium published details of the structure, function and diversity of the health human microbiome, providing a useful normal data set against which disease states can be compared.[129]
- Studies have also demonstrated that indigenous microbes are closely connected with the spread of antibiotic resistance to pathogens.[130]

3.1.8 The role of biofilms in pathogenesis

There has been increased understanding of the role of biofilms in pathogenesis, including:

- how biofilms form, through characterization of secreted proteins[131] and physical processes;[132]
- the role of biofilms in disease, for example by protecting microbes, including pathogens, from antibiotics, hydrodynamic shear and environmental challenges.[133, 134]
- the role biofilms play in horizontal gene transfer, including plasmid-borne antibiotic resistance.[135]

3.2 Detection of disease

Developments in technology have enabled faster, more accurate detection and characterisation of disease outbreaks facilitating more rapid and effective intervention. These will reduce the impact of an outbreak, regardless of its origin, thus supporting the aims of Articles VII and X of the BWC.

In particular, there have been significant advances in biosensors, biomarkers, mass spectrometry, microscopy and imaging. More comprehensive baseline data is needed to assist in comparisons and establish a 'norm' against which to compare unusual events.

We are increasingly able to differentiate between deliberate and natural outbreaks, using genomics, PCR and mass spectrometry and the emerging discipline of 'microbial forensics' can help establish attribution if a malevolent deployment is suspected (see below). Such a finding would entail obligations and commitments by other States Parties under Article VII, as well as additional agreements reached at successive review conferences.

3.2.1 Differentiating between deliberate and natural outbreaks

There have been advances in using genomics to differentiate between deliberate and natural outbreaks,[136] including:

- using genomics to differentiate between an agent of interest from background microbes, including differentiating a specific lineage of Bacillus anthacis from samples of same species and strain, offering opportunities to link an environmental sample to samples from possible production facilities;[137]
- they ability to differentiate between different isolates indistinguishable using physical characteristics enabling forensic epidemiology and modelling of the transmission routes involved;[138]
- insights into the geographic origins of agents by identifying sequence differences corresponding to geographically distant but phenotypically identical pathogens, or more broadly to the geographic distribution of genetic elements;[138, 139]
- mutational bias and possible deviations from normal evolutionary development;[138, 139]
- comparative analysis of the causative organism of an outbreak to detect anomalies in antibiotic resistance, transmission routes or in outbreak intensity and clinical dynamics which might indicate an unnatural origin.[139]

Other techniques and approaches have been used to differentiate between deliberate and natural disease outbreaks, including the analysis of agents impossible or difficult to culture,[123] including PCR[140] and mass spectrometry.[123, 140] Some of these platforms, such as PCR approaches, have been adapted for in-field use.[141]

3.2.2 Biosensors

It may be possible to detect a disease outbreak by using biosensors. These have been improved by increased sensitivity and ability to detect a wider range of agents using a wider range of samples and to detect functional changes in the host.

Improved biosensors

The capacity of biosensors to detect an outbreak has improved due to:

- increased sensitivity of the technology;[142, 143]
- applicable to a wider set of samples, for example, whole serum142 and urine;[144, 145]
- removal of the need for separate desalination and centrifugation steps in sample preparation;[146] and
- improved capacity for quantitative analysis as well as simple detection;[147, 148]
- in-field biosensors combined with mobile applications and connections to remote databases, increases their utility.[149]
- ability to detect a wider range of agents, including:
  - heavy metals;[150]
  - pathogens,[150] such as *Bacillus anthracis*[147] and the causative agents of meningitis;[148]
  - toxins;[151, 152, 153] and
  - genetic material, including oligonucleotides.[143]

Advances in biosensors have also facilitated both diagnosis and the selection of appropriate therapeutics by better detection of functional changes in a host:

- changes in phagocytes enabling differentiation between infections caused by viruses and those caused by bacteria;[154] and
- an order of magnitude increase in the sensitivity of detection of biomakers for prostate cancer.[142]

Technological advances

These improvements in biosensors are drawn from a wide range of technological advances, including:

- nanoparticles:[145, 155, 156]
  - by reducing the physical space needed for chemiluminescent studies;[154]
  - as novel signal generators;[142]
  - on chip sample preparation;[146]
  - as scaffolds to hold functional elements;[157, 158]
  - for measurements at submicron and subcellular length scales;[121] and
  - in transduction.[158]

- the design, construction and use of engineered organisms;[150, 159]
- plasmon resonance sensors;[145, 151, 153]
- planar waveguide devices;[152]
- DNA scaffolds;[160]
- the production of graphene surfaces;[161]
- quantum dots;[153, 162]
- surface enhanced Raman scattering;[148]
- amperometry and magnetic relaxation;[145] and
- synthetic biology to engineer bacteria to detect and record environmental signals in the gut[163] and probiotics for disease detection.[144]

3.2.3 Biomarkers

Biological markers of disease ('biomarkers') can detect disease rapidly. This improves our understanding of disease progression, enables earlier detection of disease, differentiates between different infections, and facilitates triaging of patients and epidemiological investigations.

Biomarkers have been identified in a range of tissues and locations, including bone marrow,[164] lung cells,[165] and human sera.[166] A synthetic system has been developed to identify biomarkers in the body and release reporters that can be detected in the patient's urine.[167, 168, 169]

Biomarkers have been used to characterize the following:

- signalling behaviour of cells;[164]
- gene products,[170] including pre-clinical indicators of infection,[171, 172] for differential diagnosis of different pathogens,[171] and interaction with drugs;[173]
- improvements in proteomics;[170]
- gene expression and protein changes caused by pathogens,[174] such as influenza[165] and *Mycobacterium tuberculosis*,[175] and to differentiate between different influenza infections;[176]
- cytokine concentrations associated with different clinical outcomes;[94] and
- previous viral infections from circulating antiviral antibodies.[166]

Biomarkers are identified and detected through a variety of different technologies, including:

- single-cell mass cytometry;[164]
- four-dimensional separation systems;[170]
- RNA-seq;[174]
- polymer microprojection arrays for transdermal biomarker detection in live animals;[122]
- RT-PCR TaqMan low-density arrays;[176] and
- nanoparticles and other carrier structures.[177]

Biomarkers are now more sensitive increasing their ability to detect rare events. This has been achieved through the use of nanoparticles;[172, 177] signal amplification;[177] and higher throughput using multiplexing techniques.[177]

3.2.4 Mass Spectrometry

Advances in mass spectrometry have contributed to our understandings of disease, facilitated detection of disease outbreaks as they occur, and identification of the agent responsible. Since the Seventh Review Conference platforms such as MALDI-TOF mass spectrometry have entered into routine use for detections and diagnosis.[178]

Mass spectrometry has been used for:

- agent identification;[123, 140, 179, 180]
- disease surveillance;[123]
- microbial forensics;[123]
- the detection of complex biological samples;[181]
- the detection of functional characteristics in pathogens, such as antibiotic resistance properties,[178] and
- differentiation between strains of a pathogen.[182]

Mass spectrometry has been combined with other novel technologies, such as:

- PCR driven Electrospray-Ionization Mass Spectrometry, enabling the identification and differentiation of agents of concern;[140, 179]
- combinations of high-throughput nucleic acids analysis and mass spectrometry for the analysis of agents difficult or impossible to culture;[123] and
- liquid chromatography–mass spectrometry for genome-wide discovery of protein-coding loci in mammals.[183]

There have also been further technical developments in mass spectrometry, including:

- ionization methodologies for high-resolution mass spectrometry, increasing the number and range of molecules that can be detected;[181, 184]
- time-of-flight approaches to mass spectrometry, which may be more suitable for the detection of biological material;[181, 184]
- miniaturization of equipment;[184]
- Matrix Assisted Ionisation Vacuum mass spectrometry and Desorption Electrospray Ionisation mass spectrometry which simplify sample preparation.[184]

3.2.4 Microscopy and imaging

Advances in microscopy and imaging have contributed to our understandings of disease, facilitated detection of a disease outbreaks, and identification of the causative agent.

Advances have enabled:

- direct observation of proteins as they conform and move;[185]
- 3D imaging on a sub-cellular level in live cells, for example mitochondria, filopodia, membrane ruffles, intracellular vesicles and mitotic chromosomes;[186]
- tracking the course of pathogens during infection of a living host;[88] and
- building all-atom models of viral capsids.[32]

These advances have drawn upon technical developments in:

- combining fluorescent and other microscopy techniques;[185]
- scanned Bessel beams used in conjunction with structured illumination and/or two-photon excitation;[186]
- bioluminescence imaging;[89]
- cryo-electron microscopy and cryo-electron tomography;[32] and
- the development of rendering opaque living tissue transparent.[117]

Ultra-cheap microscopy equipment has also become available since the Seventh Review Conference, for example paper-based microscopes costing less than $1 and disease-specific microscopes for diagnostics in severely resource limited settings.[187]

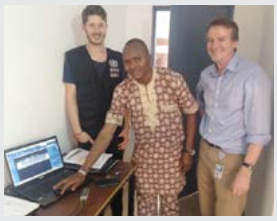
There have also been examples of being able to repurpose equipment for use in imaging, for example a commercial DVD drive was converted into a laser scanning microscope that can analyse blood and perform cellular imaging with one-micrometre resolution.[188]

3.3 Diagnosis and surveillance

Improvements in diagnostics and disease surveillance can identify causative agents more rapidly, expediting the selection of optimal treatment options and preventing transmission. These developments strengthen capacities applicable to Articles VII and X of the BWC.

Relevant improvements here include; rapid diagnosis of unknown pathogens; sequencing; PCR diagnostics; distributed diagnostics (see Box 2) and point-of-care devices; centralisation of certain types of laboratory capacity; genetic and molecular epidemiology; as well as the use of cheap and disposable equipment. Substantial improvements in diagnostic speed and accuracy were noted.

Box 2: Distributed diagnostics



Developments in genetic sequencing technologies have not only made it faster and easier to sequence a genome but have made it possible to do it around the world. The MinION nanopore sequencer was deployed by the European Mobile Laboratories in Guinea in 2015, as part of an effort to address a large scale Ebola Virus Disease outbreak. This new platform enabled Guinean authorities to obtain important diagnostic and epidemiological information within 48 hours with the samples never leaving the country.

Image:

Left: Josh Quick, University of Birmingham, UK. **Center:** Dr. N'Faly Magassouba, Infectious and Tropical Diseases Department, National Hospital Donka, Conakry, Guinea. **Right:** Prof. Miles Carrol, Public Health England, Porton Downs UK.

3.3.1 Diagnosis of unknown pathogens

Advances in metagenomics have enabled the identification of previously unculturable microorganisms,[123, 189] previously unidentified pathogens,[190, 191] and the identifications of pathogens from within mixed environmental samples.[192]

3.3.2 Bioforensics

Advances in diagnosis and surveillance can probe the origin of an outbreak and provided new and improved tools to identify, interdict and prosecute those undertaking prohibited activities (relevant to Articles I, III, IV, V and VI).

A variety of different technologies and approaches have been used for bioforensics, including:

- whole genome sequencing;[136, 137, 138, 193]
- comparative genomics;[137]
- bioinformatics;[193]
- metagenomics;[108]
- mass spectrometry;[123] and
- applications of nanotechnology.[156]

In some cases these tools have been validated for use in bioforensics, such as for high-throughput genome sequencing platforms.[194]

These technologies and approaches have increased our capacity for investigating the origins of a given pathogen by:

- linking specific phenotypic qualities of an agent with sequence data;[137]
- using sequence data to differentiate an organism of interest from background microbes;[137]
- cataloguing genomic diversity and mapping circulating mutations, providing better reference data sets;[3, 36, 193]
- use of molecular epidemiology, to trace the evolution of an agent of interest more accurately;[36]
- distinguishing between samples of a pathogen indistinguishable using traditional tests;[138, 193]
- to detect any disconnect from a natural chain of infection;[138]
- linking whole genome information to existing datasets of genetic fingerprint, previously used for pathogen identification, increasing the available reference data;[195]
- giving'insights into production methods, such as particle sizes, associated signatures, geographical origins, and intelligence information';[193]
- characterizing mixed environmental samples;[108]
- establishing the relationship between a novel agent and known pathogens;[196] and
- determining evolutionary dynamics.[196]

Research into clinical waste management has revealed that fragments of genetic material survive standard cleaning and decontamination techniques, such as autoclaving. The persistence of such material and the application of metagenomics approaches to reconstruct fragments into longer sequences, could enable the identification of an agent even after attempts to destroy all traces of its presence.[197]

Significant challenges have also been noted, including a need for a predictive capability of function from sequence data.[36]

3.3.3 Sequencing

These advances have increased the amount of benchmark and background data available for diagnostics, enabled a wider set of samples to be used in diagnostics, decreased time for confirming a diagnosis, and enabled in-country diagnostic capacities, and facilitating genetic and molecular epidemiology. As a result, sequencing technologies have been increasingly used for diagnosis and surveillance during disease outbreaks including outbreaks of H1N1 influenza,[189] *Escherichia coli*,[19, 199, 200] H5N1 influenza,[3] tularaemia,[199] meningoencephalitis,[108] malaria,[201] *Staphylococcus aureus*,[202] viral hemorrhagic fever outbreaks,[203] anthrax,[204] and wheat yellow rust.[27]

Significant challenges have also been noted, including: a need for a predictive capability of function from sequence data;[36] 'to use genomic data to develop improved tools for managing infectious diseases';[36] manipulating and analysing the data and other bioinformatics challenges;[205, 206] improving diagnostic sensitivity;[200] speeding up and simplifying workflows;[200] and further cost reductions.[200]

Developments in sequencing technology include:

- improved bioinformatics approaches and tools;[206, 207, 208, 209]
- increased speed;[207]
- reduced costs;[207]
- commercial production of novel nanopore-based sequencers;[210] and
- validation for use in investigating the use of biological weapons.[194]

New sequencing capabilities have been used for:

- cataloguing genomic diversity; [3, 27, 36, 37, 201, 207, 211, 212]
- molecular epidemiology and disease surveillance;[3, 19, 36, 136, 203, 208, 211, 213]
- understanding pathogenesis; [19, 36, 37, 202, 204, 213, 214]
- exploring metagenomics;[36, 200, 214]
- pathogen discovery and diagnostics;[19, 36, 108, 136, 199, 202, 203, 204, 205, 207, 209, 211 ,213 ,214, 215]
- therapeutics and drug discovery;[36]
- vaccines;[36]
- diagnosis of hereditary diseases;[216]
- understanding transmissibility and host range;[3, 214]
- establishing antimicrobial characteristics;[199, 201, 202, 214]
- determining infection control need;[199, 202]
- identifying agents in mixed environmental samples;[108, 208, 214, 215]
- informing clinical management;[202, 206]
- understanding host-pathogen interactions;[214]
- the identification of natural reservoirs;[207] and
- phylogenomic trees helping to explain the evolutionary relationships between pathogens, strains and clades.[217, 218]

There has also been progress in ensuring the 'backwards compatibility' of sequence data, for example, using software tools to link whole genome sequence data on pathogens with earlier pathogen 'fingerprint' data sets.[195]

3.3.4 PCR diagnostics

Using PCR assays to diagnose pathogens has proven to be 'being highly specific and cost-effective'.[212] It offers benefits over serological methods of diagnosis because fewer reagents are needed and it produces results that are more easily interpreted.[219] Automated, rapid, and sensitive PCR-platforms capable of identifying over a thousand pathogens from clinical samples now exist.[220] Cartridge-based diagnostic PCR platforms intended for use by non-specialists have been developed.[221] Some are intended for in-field use,[222] including detection of the potential use of biological weapons.[141]

Platforms using PCR techniques have been used for diagnostics since the Seventh Review Conference, including:

- real-time reverse–transcription PCR–based genotyping;[198]
- multiplexed PCRs;[223]
- multiplexed RT-PCR;[179, 224] and
- digital droplet PCR.[225]

These platforms have been used to identify a range of pathogens, including:

- differentiation between influenza subtypes and reassortants;[198, 224, 226]
- *Bacillus anthracis*,[179] including simultaneous identification of virulent and avirulent strains;[223]
- Clostridium difficile;[227]
- *Yersinia pestis*;[179]
- *Francisella tularensis*;[179]
- *Brucella* spp.;[179]
- *Burkholderia spp.*;[179] and
- *Rickettsia prowazekii*.[179]

Alternative approaches using different techniques have been developed, for example:

- isothermal amplification techniques offers similar information to the thermal heating traditionally used in PCR but at a reduced cost[220, 227, 228]
- loop-mediated isothermal amplification (LAMP) technology has streamlined DNA extraction and amplification steps.[227]
- PCR technologies are also more portable, for example through integration of sample preparation and data analysis steps into a single machine and through approaches reducing or removing energy requirements.[227]

- using PCR to quantify the amount of pathogens present, rather than simply detect their presence, for example, digital droplet PCR has been used to quantify viral titer with better accuracy than traditional methods for establishing Ct values but with labour intensive purification steps, as an absolute quantification (negating the need for comparison against an external standard), and as part of a fully automated system.[225]

3.3.5 Distributed diagnostics
(including point-of-care diagnostics)

Since the Seventh Review Conference, there has been a trend 'to make sophisticated tests, or assays, more easily performed with less training, leading to decentralization and diagnosis closer to the point-of-care'.[229] Their value in combating high-impact infectious diseases has been noted, as rapid diagnostics and treatment here is critical.[230] The application of nanotechnology has led to improved accuracy, increasing speed and reductions in costs of point-of-care diagnostics.[231] Integration with modern information technology platforms, such as smart phones, has also enabled a more distributed approach towards diagnostics.[232]

Point-of-care diagnostics

There is global interest in point-of-care diagnostics with new products being developed in every region of the world.[230] The cost of certain point-of-care diagnostic tools has fallen since the Seventh Review Ronference.[229] Point-of-care tests have been developed specifically for biological weapon agents.[230]

Developments in point-of-care diagnostics include, the ability to detect:

- differentiation of bacterial and viral infections;[154]
- all strains of H5N1 influenza;[233]
- dengue fever;[233]
- chikungunya;[233]
- hand, foot and mouth disease;[233]
- malaria;[233]
- tuberculosis;[234] and
- the goat disease Contagious caprine pleuropneumonia.[222]

Lab-on-a-chip technologies have continued to develop, for example, including for the detection of antibiotic resistance characteristics in pathogens.[235]

Capabilities for point-of-care diagnostics based upon analysis of breath have been demonstrated since the Seventh Review Conference.[231]

Point-of-care diagnostics have also been used for the optimization of drugs and medication, thereby influencing clinical management decisions.[230]

Tools

A number of technical developments have contributed to advances in point-of-care diagnostics since the Seventh Review Conference, for example:

- the use of nanoparticles to significantly reduce the physical space needed for chemiluminescent studies;[154]
- capillary driven and paper-based microfluidics;[236]
- multilayer soft lithography;[236]
- multiphase microfluidics;[236]
- electrowetting-on-dielectric driven droplet microfluidics;[236]
- electrokinetics;[236]
- centrifugal microfluidics;[236]
- the development of bio-based polymer beads;[237]
- the use of cell phones to access remote data sets and analysis;[149, 232]
- Mass/Piezoelectric detectors';[234]
- field portable versions of classical ELISA (using lateral flow chromatography; [234]
- advances in PCR technologies to make it more readily adapted to portable and in-field use;[234]
- microfluidic technologies with integrated sensors for processing bodily fluids such as blood or saliva;[238]
- the creation of paper-based gene circuits;[231] and
- the application of synthetic biology approaches to developing probiotics for the diagnosis of disease.[144]

3.3.6 Laboratory capacity

Whilst certain diagnostics are becoming more distributed, 'more advanced (and likely more expensive) diagnostic tests may require specialized equipment, resources and laboratories (e.g. reference laboratories)' as a result, there has also been a trend towards the 'consolidation of testing in larger labs, driven by economies of scale, availability of expertise, and the development of newer diagnostic approaches that have substantial complexity and costs'.[239]

3.3.7 Genetic and molecular epidemiology

Enhanced disease surveillance capabilities now plays an important role in dealing with disease outbreaks, such as the 2009 H1N1 influenza pandemic.[240] Advances in gene sequencing technologies are increasingly used in epidemiology. [36, 241]

Sequence data has been used to:

- gain insights into the geographic origins of pathogens;[19]
- demonstrate that the geographic distribution of pathogens is wider than previously thought;[242]
- map the current circulation of mutations known to affect certain phenotypic implications, such as enabling mammal-to-mammal transmission[3] or antibiotic resistance;[218]
- identify the most feasible epidemiological explanation of a diseases outbreak;[138]
- determine genetic diversity amongst geographically or temporally disparate pathogens;[27, 243, 244]
- determine the relationship of a novel pathogen to known relatives;[196]
- to study the emergence and spread of antibiotic resistance;[245] and
- confirm infection and transmission events.[203]

Sequence data has also been connected to phenotypic characteristics to determine whether transmission is a result of specific characteristics of the pathogen, or other factors such as vector dynamics and host susceptibility.[246] There have been significant advances in analysis and interpretation of results[209] and greater accuracy in the detection of genetic elements associated with virulence and drug resistance since the Seventh Review Conference.[37]

Other advances, such as those in mass spectrometry,[123, 182] have improved the capacity for molecular epidemiology.

Genetic and molecular epidemiology have been used to investigate outbreaks of diseases since the Seventh Review Conference, including:

- *Escherichia coli*, to provide insights into the origins of the bacteria and core aspects of its pathogenicity;[19]
- *Yersinia pestis*, enabling comparative analysis of factors such as pathogenicity with other currently circulating and historical strains;[246]
- *Staphylococcus aureus*, to characterise genome stability and the independent acquisition of disease characteristics in different locations;[247]
- MERS-CoV, including for evolutionary mapping;[196]
- four viral hemorrhagic fever outbreaks, to identify and confirm infection and transmission events;[203]
- wheat yellow rust, to track phenotypic changes and map the evolution of the pathogen throughout a country;[27] and
- evolutionary mapping of Peste des Petits Ruminants Virus[217] and Ebola Virus Disease.[248]

Current shortcomings include the lack of background or normal data.

Progress has been made in identifying the limits of and strategies for improving the global mapping of infectious diseases of clinical significance to humans.[249]

3.3.8 Cheap and disposable equipment

Diagnostic equipment, such as PCR, more suitable for use in resource limited settings has been developed by, for example removing the requirement for electricity, replacing traditional heating elements with chemical components taken from hand and boot warmers.[250] Similarly, cheap, paper based microscopes have been developed with different levels of resolution and for specific diseases.[187]

3.3.9 Diagnostic speed and accuracy

Improvements in whole genome sequencing and its falling costs have led to more widespread use of the technique in diagnostics and epidemiology. It is faster and more accurate that other approaches[251, 252] and has proven particularly useful in differentiating between closely related pathogens.[224]

The extraction of pathogenicity-related information from sequence data; a process which used to take weeks, can now be accomplished in a day.[253]

There has also been progress in developing high-throughput approaches to diagnostics, for example the use of Luminex microarray hybridization to identify influenza subtypes.[254] Progress has also continued in lab-on-a-chip platforms including in terms of speed, versatility and user friendliness.[235]

Synthetic biology is reducing the time needed to produce specific antigens or antibodies for use in diagnostics tests.[252]

However, shortcomings continue to persist. For example, a lack of quality assurance in toxin diagnostics has been demonstrated internationally.[78]

3.4 Preventing, mitigating and treating disease using vaccines and drugs

The timely use of effective vaccines and therapeutics prevents or reduces the impact of outbreaks, regardless of cause. This is of direct relevance to Articles VII and X. Rapid detection and characterization of infectious agents reduces the time required to develop vaccines, drugs and other counter-measures.

Outsourcing of key production steps has reduced the need for dedicated vaccine production infrastructure. It is increasingly simpler, faster and cheaper to industrialize production processes. Single-use equipment and modular production technologies shorten turn-around times. A more distributed production base in industry reduces the distance a product has to travel to its point of use. However, regulatory and liability issues associated with diagnostics, drugs and vaccines in health emergencies continue to limit potential for progress and this is an issue that should be addressed.

High-throughput platforms and 'big-data' approaches continue to reveal a wealth of potential new targets and candidates for drugs and vaccines. The design, testing and optimisation processes have been streamlined by the 'digitalization' of biology supported by better computational technologies, improved capacity for rational design, integration of synthetic biology approaches, more sophisticated modelling tools, enhanced synthesis technologies and a wider array of platform technologies (see Box 3).

Online laboratories and facilities now offer all of these services at a single site, as an 'organism design support service'. Throughput has been improved using machine-learning and semantic web-based approaches. Enhanced screening of drug candidates and other enabling tools have helped streamline the development pipeline. However, bioinformatics capabilities remain a major challenge in this area.

Since the Seventh Review Conference, a number of new drug classes have been developed and existing classes of drugs have been further exploited. Examples include: antibody-based drugs; novel drugs for diseases traditionally associated with biological weapons (e.g. anthrax, ricin); the use of drug combinations; drugs to target disease vectors; and in identifying useful off-label indications for existing drugs.

Vaccines have been developed for combating multiple agents or strains by targeting conserved regions and rendered more effective and stable through the use of virus-like particles and improved adjuvants. A number of new avenues for designing vaccines have been explored, utilising synthetic biology, DNA nanostructures and RNA viruses.

Box 3: An example of how the digitization of biology accelerates vaccine development.
The Novartis H7N9 influenza vaccine response – Combining synthetic virus generation with flu cell culture platform

Synthetic virus rescue in collaboration with Synthetic Genomics Vaccines Inc. Diagram reproduced with permission from Novartis.

3.4.1 Drug development

A range of new techniques has improved the development of existing drugs and opened up opportunities for further discovery.

High throughput systems

High-throughput platforms continue to provide a wealth of new information on potential new targets for drugs,[255] including sequencing[36, 256] and toxicological screening.[257, 258]

Connecting such data to biological function remains a significant challenge,[36] although advances have been made in developing model systems can now recapitulate *in vivo* viral life cycles.[18]

Computational and bioinformatics

Computational and bioinformatics approaches have improved design strategies, including:

- designing proteins that bind a surface patch of interest on a target macromolecule;[259, 260]
- data-driven prediction of drug effects and interactions;[261, 262]
- prediction of novel drug-drug interactions using databases,[263, 264, 265] machine learning,[266] and semantic web-based approaches for data mining;[267]
- development of tools for extracting machine-usable data from past publications;[268]
- computational design capabilities to stabilize enzymes;[269] and
- integration of multiple data sources and 'big data' approaches to drug discovery.[270]

Synthetic biology

Synthetic biology approaches have also been applied to drug discovery[271] and have helped identify candidates for: new antibiotics, drugs to reverse antibiotic resistance, anti-diabetes drugs, and immunosuppressants; as well as cancer therapies, including bacterial synthetic devices, viral synthetic devices, and a transformation sensor.[272]

Synthetic biology approaches have resulted in strategies for controlling and measuring cell-environmental interactions in vitro, including strategies for high-throughput analysis.[273]

There has also been progress in developing chassis for clinical applications.[274] Since the Seventh Review Conference, platforms offering remote support for designing, compiling, trouble shooting and optimizing custom biological organisms have appeared, for example with industrially focused, large scale platforms for the synthesis of genetic material.[275]

Handling and manipulating material has been improved, for example: the identification of a protein for purifying, immobilizing, and detecting all types of human and nonhuman immunoglobulin G' antibodies;[125] and developments in rational enzyme design.[271]

Enhanced screening of drug candidates has streamlined the development pipeline and other tools important for drug development. Examples since the Seventh Review Conference include, a protocol for testing the ability of a specific protein to 'rescue' knock-out strains of bacteria[276] and high-resolution dose – response screening using droplet-based microfluidics.[277] Fully automated, remotely accessed laboratories have also emerged.[278]

Improved tools to explore important features of the target and host have been developed, for example:

- a chip based method for producing uniform vesicles facilitating research into proteins in cell walls, common drug targets;[289]
- genome-scale RNA interference target sequencing to reveal the transporters, organelles, enzymes and metabolic pathways that function to facilitate drug action;[280]
- experimental-computational technology for inferring network models that predict the response of cells to perturbations;[281] and
- mice optimised for use with genome editing technologies.[282]

3.4.2 Novel drugs

A number of new drug classes have been developed since the Seventh Review Conference, including:

- mRNA-based therapeutics;[283]
- antibody-drug conjugates,[271]
- nanoparticle-based drugs;[271]
- proof of principle studies on using synthetic biology techniques to engineer microbes to sense and eradicate pathogens;[284, 285]
- nanoparticle-based drugs able to interact with viruses inside infected cells; [286]
- the use of CRISPR/CAS9 to edit human genetic components linked with disease[286] as cancer immunotherapies, and cell-based therapies for HIV, primary immune deficiencies, and autoimmune diseases.[287, 288]

Drug classes identified at previous review conferences are still yet to reach full development but are improving, for example, advances in developing siRNAs-based drugs[289, 290] have improved stability, potency, reduced off-target effects, and increased efficient delivery and led to the development of therapeutics in Phase I and Phase II clinical trials.[291]

Progress has been made in developing antibody-based drugs, against:

- bacteria, such as *Bacillus anthracis*;[292, 293, 294]
- toxins, such as Ricin[70, 74, 75, 76] and Abrin;[59]
- viruses, such as Ebola viral[295] and
- H1N1 influenza.[296]

The FDA licensed the first monoclonal antibody against inhalational anthrax since.[297] Additional antibody therapies for inhalational anthrax have subsequently been licensed.[294]

Since the Seventh Review Conference a range of new drugs and drug candidates have been developed, including to target:

- *Bacillus anthracis*;[291, 292, 293, 294, 297, 298]
- toxins, such as Botulinum neurotoxin,[56] Ricin,[58, 68, 70, 71, 72, 74, 75, 76] and Abrin;[59]
- pathogenic viruses, using interferons with enhanced activity;[299]
- Ebola virus;[300]
- biofilms;[133]
- prion-based diseases;[83, 301]
- dengue virus;[302]
- H1N1 influenza;[296]
- Margburg virus;[289]
- H7N9 influenza;[28] and
- genetic factors known to influence disease in humans.[286]

Drugs have also been used in new combinations to increase efficacy. For example, research has demonstrated the value of administering both antibodies and antibiotics in treating anthrax.[293, 298]

There has also been progress in developing drugs to target important disease vectors, for example the Wolbachia bacteria[303] and mosquitoes[120, 304] important in malaria.

There has also been progress in identifying opportunities to use licensed drugs to treat other diseases, for example, to treat prion diseases,[83] and H7N9 influenza.[28]

3.4.3 Vaccine design

The efficiency and speed of vaccine development has improved through a combination of stored datasets, design and techniques.

This included the use of:

- large quantities of genetic sequence data that provide many novel targets for vaccines;[36]
- improved understanding of pathogenicity and host-pathogen interactions, thereby assisting vaccine design;[305, 306]
- improved computational design tools, for example in HIV vaccine design[307] and structure based antigen design;[308]
- rational design and production tools,[307, 308, 309, 310] in particular in bio-based processes[310] and the ability to synthesize vaccine candidates from manipulated sequence data.[312]

There have been attempts to develop new approaches to designing vaccines:

- advances in synthetic biology have been applied to vaccine development, including production of antigen-producing immunostimulatory liposomes as genetically programmable synthetic vaccines[313] and development of a methodology for synthetic attenuated virus engineering;[314]
- the use of DNA nanostructures as platforms for vaccines;[312]

- the use of synthetic drug-like small molecules to inhibit viral infection of cells;[315] and
- the use of RNA viruses to produce vaccine antigens.[316]

Notable progress has been made in the development of more effective and stable vaccines, such as through the use of virus-like particles, for example:

- synthetic capsids that confer immunity and offer significant benefits over live agent, or inactivated agent vaccines for diseases such as Foot-and-Mouth Disease;[312, 317, 318]
- self-assembling influenza nanoparticle vaccines that elicit broader and more potent immunity than traditional influenza vaccines;[312, 319]
- the application of engineering principles for rational design;[309] and
- the construction of virus like particles to confer immunity against H7N9 and H7N3 influenza viruses,[320] and Hepatitis E and related diseases.[321]

Existing approaches to increase impact and efficacy continue to be developed, for example:

- conjugate vaccines;[311]
- the use of baculovirus expression systems;[306, 308, 322]
- better targeting of conserved regions;[296, 307, 312, 323]
- use of mammalian cell culture production and synthetic vaccine seeds;[324]
- reduced reliance on generating sheep antisera;[324]
- better adjuvants;[306, 308, 324, 325, 326] and
- novel nucleic acid vaccines,[308] including mRNA vaccines.[327]

New or improved vaccines have been designed and developed to prevent:

- chikungunya virus;[313]
- *Staphylococcus aureus*;[313]
- Foot-and-Mouth Disease virus;[317]
- Ebola virus;[315, 328]
- influenza viruses;[310, 320]
- *bunyaviruses*;[315]
- *arenaviruses*;[315]
- *paramyxoviruses*;[315]
- *coronaviruses*;[315]
- *flaviviruses*;[315] and
- Hepatitis E.[321]

3.4.4 Vaccine and drug production

The space and resources required for biologics production has also decreased and the physical size of production equipment has been drastically reduced. Smaller facilities using more compact equipment increases the range of potential sites and reduces logistical challenges, in some cases also offering cost benefits. Scaling-up production to industrial levels has been simplified and can be accomplished more quickly, although it can still take years.

In some cases, the costs of industrial scale-up have fallen, through improved 'directed evolution' techniques for example. In other cases, improvements in efficacy and efficiency, such as those associated with automation and miniaturization, may incur increased costs.

Bio-based production and biosynthesis have become common methodologies, aided by particular developments in the use of yeast 'chassis' and 'scaffolds' to control the spatio-temporal arrangement of components. There have been improvements in vaccine expression, in particular through insect cell line and suspended cell cultures and the use of bulk production material.

The increase in usage of disposable or single-use equipment has also been noteworthy. The range of processes for which disposable equipment is available has increased in number and complexity and increasing standardization of parts, facilitates switching to disposable equipment.

Outsourcing of production, including the advent of 'biofabs', has also become a reality since the Seventh Review Conference. Post-production purification steps have been improved or simplified, as a result of the growing regulatory attention paid to ensuring viral removal or inactivation.

Reduced size of production facilities

The space required for drug and vaccine production has decreased. Examples since the Seventh Review Conference include:

- the use of suspended cultures in vaccine production;[329, 330]
- the use of egg-free systems in vaccine production;[331]
- industrial use of microreactors for chemicals and small biological;[332]
- baculovirus/insect cell expression systems to produce recombinant proteins and vaccines;[329, 333] and

- the potential to use yeast-based systems for opinoid production.[334, 335]

The physical size of production equipment has been drastically reduced. Examples since the Seventh Review Conference include the use of suspended cultures in vaccine production[329, 330] and industrial use of microreactors for chemicals and small biological.[332]

Smaller facilities using smaller equipment increases the range of potential sites and reduces logistical challenges, in some cases also offering cost benefits.

Quicker simpler production

Improvement in the synthesis of genetic material and other synthetic genomics techniques has enabled the outsourcing of biological production, for example, vaccine candidates.[336, 337] Multipurpose biological production facilities have been used for outsourced production, for example for the industrial production of a precursor for an anti-malarial drug.[338]

Disposable equipment has been used in the production of vaccines,[339, 340, 344] monoclonal antibodies,[341] recombinant proteins,[341] and are being developed for use with stem cells and personalised medicine.[341]

The time required to scale up processes for large-scale production has fallen in some cases, for example, increased automation and miniaturization of development processes has occurred.[342] Whilst these processes happen faster, they can still take years.[336]

Certain scale-up processes have been simplified. Examples since the Seventh Review Conference include: the use of directed evolution to remove reliance on animal serum;[329] the improved engineering of strains prior to scale up;[338, 342] and increased use of virus-like particles instead of viruses.[321]

There have been improvements in microfluidic reaction vessels used for drug production, for example, there have been advances in flow microreactors since the Seventh Review Conference.[332]

The range of processes for which disposable equipment is available has increased in number and complexity. Examples since the Seventh Review Conference include: tangential flow filtration;[343] mixing and perturbing materials;[343] and downstream processing equipment.[339, 340]

There has been increasing standardization of parts, making it easier to switch to disposable equipment including 'standardized, off-the-shelf assemblies for cell culture harvest and tangential flow filtration, disposable, aseptic, genderless, universal connectors, and non-proprietary films and materials of construction for its bags, connectors, and tubing'.[343]

Purification steps have been improved or simplified and there has been growing regulatory attention paid to ensuring viral removal or inactivation.[344] Examples since the Seventh Review Conference include:

- the improved use of directed evolution;[329]
- multimodal chromatography;[345]
- increased automation and miniaturization;[342]
- the use of UV-C irradiation for viral inactivation;[346] and
- research to determine the comparative efficacy of different viral inactivation techniques.[347]

Cost of production

Certain costs associated with industrial scale-up have fallen, for example, through improved directed evolution.[329] In other cases, improvements in efficacy and efficiency have increased costs. Examples since the Seventh Review Conference include: increased automation and miniaturization of development processes.[342]

Biobased production and synthesis

Bio-based production and biosynthesis have become more common methodologies for vaccine and drug production,[348] including uses for:

- new secondary metabolites with novel therapeutic activities, such as complex polyketides, halogenated and alkaloids;[349]
- hybrid materials for drug delivery;[349]
- toxins;[350]
- the industrial scale production of terpenoids;[348]
- peptides;[348]
- co-stimulators of immune responses for use with vaccines;[351]
- opinoids;[352] and
- delivery devices for vaccines.[351]

There have been significant developments in efforts to build designer yeast since the Seventh Review Conference. Such a yeast might offer opportunities for enhanced chassis for bio-based production and biosynthesis. Recent developments have included:

- the use of tags enabling the differentiation of synthetic components from wild type counterparts, especially the development of a real-time PCR assay for their detection;[353]
- an *in vivo* method for assembling designed DNA fragments;[354] and
- a novel genetic assembly system for compiling expression pathways.[355]

Scaffolds made of various materials have been used to increase the efficacy of production processes by controlling the spatio-temporal arrangement of components, including DNA scaffolds and nanostructures[160, 356, 357] and proteins.[358] Scaffolds have also been used to enhance the efficacy of the production of different vaccines and drugs, for example, resveratrol;[356] mevalonate;[356] and antigen-adjuvant complexes.[329]

Recombinant plants and animals

There have been improvements in the use of recombinant plants and animals to produce vaccines and drugs.

Plants have been used to produce:

- a treatment for Gaucher's disease grown in carrot cells;[359]
- a vaccine candidate for H1N1;[360]
- monoclonal antibodies to treat Ebola;[361, 362]
- antigens for rabies;[363]
- antigens for hepatitis B;[363]
- antigens for measles;[363]
- antigens for avian influenza;[363]
- antigens for anthrax; [363] and
- antibodies to bovine viral diarrhea virus.[363]

Animals have been engineered to produce recombinant proteins and vaccine candidates.[363]

Tools

There have been improvements in the tools enabling the controlled design and production of biological materials. Examples since the Seventh Review Conference include transient gene expression in serum-free suspension-growing mammalian cells for the production empty viral capsids,[330] as well as progress in developing standardized biological parts and devices.[349] Advances have also simplified the bulk production material.[364]

There have been developments in vaccine production, for example:

- the application of recombinant DNA technologies to the development of conjugate vaccines;[311] the use of synthetic genomics approaches to 'print' vaccine candidates;[336, 337, 340]
- the creation of virus-like nanoparticles that elicit broader and more potent immunity than traditional vaccines;[340] and
- the development of module designs for backbones using optimised backbones and key structural motifs from the virus.[365]

Different expression systems used since the Seventh Review Conference include:

- the use of insect cell lines;[322, 329, 333]
- the use of human cell lines;[329]
- the use of suspended cultures;[329, 330, 340]
- the use of egg-free systems;[331]
- the use of chemical peptide synthesis;[340] and
- the use of transgenic plants and animals.[340]

3.4.5 Vaccine and drug delivery

Significant hurdles to storing or shipping labile therapeutics have been overcome. There have been notable successes in replacing cold chains and increasing the environmental stability of vaccines and drugs. In some cases this enables room temperature storage, for example, using alternatives to live agents such as the use of empty viral capsids.[317]

Automated design strategies and other tools have made it easier to engineer and tailor drug delivery systems. The range of drug delivery platforms, such as enhanced and novel viral vectors and 'microneedles' has increased. Trans-dermal delivery systems are more effective and the range of substances that can be successfully delivered this way has increased since the Seventh Review Conference, opening opportunities for the non-invasive use a wider range of drugs and vaccines. There have also been improvements in targeted delivery systems, ensuring enhanced drug or vaccine access to the desired sites, tissues or cell-types following administration.

Elucidation of nanoparticle structure-function relationships has led to improved drug delivery vehicles. The range of nanoparticle-based drug delivery platforms has increased and now includes formulations that can cross barriers and penetrate previously unreachable sites. Nanoparticles can now be designed to enhance the activity of their payload or to overcome its rapid metabolism. This reduces costs and increases efficiency by reducing the amount of payload needed, reducing threshold activity requirements for effective payloads, or prolonging their action be extending their effective life time. Controlled release of payloads, for example by remote activation or environmental response, reduces side effects.

Vaccines

A number of different approaches have been developed to increase the environmental stability of vaccines and drugs and, in some cases enable room temperature storage, including:

- self-standing silk protein biomaterial matrices to stabilize labile vaccines and antibiotics up to temperature of 60°C for over 6 months;[366, 367]
- coating viral capsids with a mineral coat, or other materials to store a vaccine at 26 °C for more than 9 days and at 37 °C for approximately 1 week;[312, 368]
- engineering structural changes into viral capsids to improve thermal stability.[312, 318]

Drug delivery vehicles

Improvements have also been made to drug delivery vehicles, such as nanoparticles, to make them more environmentally stable.[369]

New tools to engineer and tailor drug delivery systems have been developed:

- applications of synthetic biology to construct biohybrid delivery materials;[349]
- design guidelines;[370]
- more sophisticated ways to fabricate nanostructures;[371]
- the ability to build larger nanoparticles;[372]
- more refined controlled release;[372, 373, 374]
- enhanced drug loading;[372]
- the ability to build nano metal oxide frameworks;[372]
- the ability to self assemble particles from increasing numbers of sub-units;[375]
- self assembling (and disassembling) systems;[376]
- collocation of drugs, ligands and other functionalities;[377]
- highly automated design strategies for complex nanostructures;[378] and
- design guidelines to increase the penetration of targeted nanoparticles.[379]

The range of drug delivery platforms has increased since the Seventh Review Conference. Examples include:

- the use of lipid-based liquid crystalline nanoparticles;[380]
- magnetic core-shell nanoparticles for aerosol drug delivery; [381]
- cubic phase nanoparticle for sustained release of ibuprofen;[382] and
- self-assembled liquid crystalline nanoparticles.[383]

Viruses have been used to deliver novel drugs, including CRISPR/CAS9 elements.[384] Improved viral vectors for gene therapy have been identified, including the synthesis of ancestral Adeno-associated virus vectors.[385]

Trans-dermal delivery

There has been notable progress in trans-dermal delivery:

- more effective microneedles[366, 386, 388, 389] and characterization of kinetics to improve their efficacy;[390]
- skin patches;[366, 391, 392]
- electroporation;[387, 393]
- low-frequency sonophoresis;[387]
- iontophoresis;[387] and
- tattoo delivery patches.[394]

The range of material that can be successfully delivered through the skin has also been expanded since the Seventh Review Conference, opening opportunities for the non-invasive use a wider range of drugs and vaccines. Transdermal delivery can now deliver:

- DNA vaccines;[386]
- immune-stimulatory RNA;[386]
- biodegradable polycations;[386]
- the co-delivery of DNA and siRNA;[126]
- proteins;[387]
- inactivated virus vaccines;[395] and
- insulin.[392]

Considerable progress has been reported in the manufacturing methods used to produce transdermal delivery devices, in particular microfabrication techniques in silicon, metal, glass and polymers.[396]

Cellular delivery

Advances have also been made in delivering materials into cells, for example, biodegradable silicon nanoneedles.[126, 397]

There have also been developments that enable more precise targeting of the agent:

- improved control over the placement of targeting ligands and nanoparticles and carriers;[377]
- improved capabilities to design and build antibody-drug conjugates;[398]
- engineered biomaterials for targeting;[351]
- the development of polymeric nanoparticles for targeting;[399]
- neurological targeting,[364] targeting based upon genetic differences, such as SNPs;[400]
- active and passive targeting;[401]
- targeting to specific organs or cells;[374, 385, 400, 403]
- cell-type-specific targeting for siRNA-carrying nanoparticles;[399] and
- communication between nanoparticles to improve targeting.[404]

Nanoparticle delivery vehicles

An increasingly sophisticated understanding of the structural-function relationship of nano-particles has improved their use as drug delivery vehicles.[370, 405, 406]

Progress has occurred since the Seventh Review Conference, with a July 2011 review noting they often fail to meet clinical expectations but progress was being made in re-engineering natural carriers with specific functions vivo that could be used as drug delivery carriers.[407]

By May 2012, a review noted nanoparticle carriers could be used to ' increase the therapeutic index of many components' and listed a limited number that were already on the market or in phase III trials.[312]

And April 2014 a review noted that 'advanced drug delivery systems based on micelles, polymeric nanoparticles, and dendrimers' and that 'polymeric carbon nanotubes and many others demonstrate a broad variety of useful properties'.[408]

The range of nanoparticle-based drug delivery platforms has increased and now includes those designed to cross boundaries and penetrate previously unreachable sites:

- enhanced tissue penetration;[409]
- a nanoparticle carrier designed to deliver siRNA through the skin;[376, 410]
- delivery across the blood-brain barrier;[374, 411, 412] and
- nanoparticles designed to penetrate cell membranes.[286]

Chemical cofactors can be used to increase the uptake of proteinaceous drugs.[413]

Nanoparticles can now be designed to overcome specific challenges, such as: overcoming low oral absorption rates;[383] overcoming rapid metabolization of drugs;[381, 377, 383] improved activity;[372, 377, 411, 413] reduced toxicity;[370, 372, 374] and extended time circulating in the blood by reducing clearance by the liver.[402]

This reduces costs as well as hurdles in drug and vaccine design by enabling successful vaccination using less material, or material with lower activity, or which would normally be removed too quickly to have an effect.

Notable progress has been made in controlling the release of payloads, further increasing efficacy and reducing side effects:

- nanoparticles controlled by ultraviolet light;[409]
- a remotely activated protein-producing nanoparticle;[414]
- aerosol optimised delivery nanoparticles;[376, 381]
- environmentally-responsive nanocarriers;[376, 403, 406, 415]
- biodegradable nanoparticles;[406]
- delivery platforms intended to circumvent the immune system.[374, 403]

Nanoparticles have also been used as delivery devices for viral antigens to stimulate an immune response[366] as well as co-stimulators for vaccines to help increase the immunological response[393] and establish immunological memory.[351]

3.4.6 Antimicrobials and drug resistance

Past advances in treating disease are being reversed by the increasing antimicrobial resistance. For example, antiviral resistance and resistance to anti-malarial therapies have been growing in both numbers and geographic distribution.[416, 417]

However, there is now a better understanding of the mechanisms involved, including the identification and characterization of genetic components, structure-function relationships, metabolic processes, and community responses. Some new drugs have been developed, including additional antimicrobials (comprising both antibiotics and antivirals), therapies to re-sensitize microbes to existing antimicrobials, and drugs to target persistent organisms in biofilms or resistant cells.

Understanding resistance

Notable progress has been made in understanding the mechanisms involved. There has been progress in the identification and characterization of genetic components associated with antimicrobial functions and drug resistance, including the mutations responsible for the following:

- artemisinin resistance in *Plasmodium falciparum*;[418]
- carbapenem resistance in *Enterobacteriaceae*,[419] *Pseudomonas aeruginosa*,[420] *E.coli*,[421] and *Acinetobacter* sp.;[422]
- methicillin resistance in *Staphylococcus aureus*);[245]
- neuraminidase-inhibitor resistance or reduced sensitivity in influenza viruses,[423] including neuraminidase-inhibitor resistance in H7N9 influenza virus without loss of *in vivo* virulence or transmissibility;[12]
- vancomycin resistance in *Staphylococcus aureus* and enterococcal bacteria[424] and *Staphylococcus aureus*;[425, 426] and
- oseltamivir resistance in influenza A viruses.[427]

Understanding how key structural elements affect antimicrobial functions and drug resistance has been elucidated through study of the following:

- structural components used for exchanging plasmids, including those containing anti-microbial resistance factors;[29]
- how biofilms protect pathogens from antibiotics;[428]
- differences of neuraminidase structure on the efficacy of inhibitors;[423]
- how the structure of the M2 ion channel impacts the antiviral drugs amantadine and rimantadine;[429, 430]
- how Influenza A polymerase binds to the viral RNA promoter;[431] and
- the mechanism for artemisinin resistance in *P.falciparum*.[432]

There has also been progress in understanding bacteria metabolic responses to sensing antibiotics in their environment and the range of responses triggered[433] and the mechanisms that pathogen 'communities' use to become resistant to antimicrobial therapy, for example the use of dormant persister cells in *Mycobacteria*,[434] *E.coli*,[435, 436, 437] *Burkholderia pseudomallei*[438] and associated mechanisms.[345, 435]

Particular progress has been made in understanding of the interconnections between pathogenicity, transmissibility and drug resistance, for example specific mutations that can confer additional functional characteristics without degrading others.[12]

More sophisticated tools for identifying factors associated with antimicrobial function and drug resistance have been developed, for example:

- improved understanding of how antibiotic resistance spreads in the wild, such as by horizontal gene transfer;[38, 135]

- improved integration of sequence and structural analysis for more effective identification of genetic elements connected with drug resistance;[37]
- of CRISPR/CAS9 genome editing tools;[439]
- microfluidics;[345]
- reporter genes;[345]
- transcriptomics;[432] and
- the recreation of a centralised repository for pathogenicity islands and resistance islands.[43]

Pathogens with increased antimicrobial properties and drug resistance have been produced, including:

- Salmonella;[440]
- *Bacillus anthracis*;[440]
- *Escherichia coli*;[440]
- *Vibrio cholerae*; [440] and
- *P. falciparum*.[439]

New drugs and drug targets

A limited number of new antimicrobial drugs and drug targets have been identified. A December 2014 review of antibiotics under development at the time, estimated that 37 candidates were in clinical development.[441] This number had fallen from 45 candidates in February 2014.[442]

An April 2015 review of novel influenza therapies highlighted 'several new classes of antiviral candidates targeting viral replication through individual domains of the polymerase and the nucleoprotein have been developed through structure-based design'.[443]

Novel antivirals identified since the Seventh Review Conference include:

- a novel mechanism for inducing apoptosis in cells containing viral dsRNA;[444]
- novel M2 ion channel targeting antiviral drugs that are unaffected by known resistance mechanisms;[429, 445, 446, 447]
- a novel antiviral drug which inhibits the cellular entry of many lipid-enveloped viruses;[448]
- the discovery and synthesis of novel benzofurazan derivatives which inhibits influenza A viruses;[449]
- a novel viral RNA polymerase inhibitor, Favipiravir which blocks the replication of a broad range of RNA viruses;[450]
- optimized small-molecule inhibitors of Influenza Virus Polymerase, which target new locations distinct from known antiviral resistance mechanisms;[451]
- potential drug targets identified as a result of the structural characterization of Influenza A polymerase binding to the viral RNA promoter;[431] and
- a hybrid small molecule capable of disrupting the function of Influenza A polymerases.[452]

Novel antibiotics identified or under development since the Seventh Review Conference include:

- compounds that resensitise multidrug resistance bacteria by blocking efflux;[453, 454]
- a novel class of antibiotics that remove their ability to do harm, rather than kill bacteria;[455]
- characterization of the antibiotic properties of silver;[456]
- a novel antibiotic from a marine-derived Actinomycete that demonstrated efficacy in killing both gram-positive and gram-negative bacteria;[457]
- reinvigoration of research into bacteriophage-therapy, including purified lysins;[454]
- quorum-sensing inhibitors;[454]
- small molecule inhibitors of multi-drug efflux pumps;[454] and
- nanoparticles that can kill bacteria.[454]

Examples of other therapeutics identified since the Seventh Review Conference include:

- disruption of biofilms[428] including the use of motile bacteria exuding anti-bacterial factors;[133]
- an adjuvant that resensitises P. falciparum to artemisinin;[417]
- the identification of an FDA-approved anti-cancer drug which eradicates 'persister' cells;[458] and
- photodynamic therapy.[454]

3.5 Responding to, rolling back, and recovering from disease

The speed at which an outbreak can be terminated and normal life resumed, determines the overall impact of a disease event. These are important considerations for both Articles VII and X of the BWC.

Given the advances in science in technology, and provided that the remaining logistical, economic and technical challenges can be surmounted, it should now be possible to assemble patchwork capabilities into a diffuse but integrated system for countering global or local outbreaks. Obviously this highly desirable objective would only be successful if backed by political will and the fostering of international support and collaboration.

Such a system could scale from local needs through to international responses. A structure that enabled data, such as pathogen sequences, to be shared more effectively and efficiently would facilitate a rapid an effective response. As expertise and 'know-how' matures, opportunities for technological leapfrogging appear, as was the case with mobile communication systems. Developing countries can then access opportunities and capabilities in the field that match, if not surpass, those found in developed countries.

A co-ordinated response to a disease outbreak can only be effective if the communities involved are prepared to work with the emergency responders. Anthropological studies have identified and developed 'good practice' guidelines for obtaining community cooperation. Equally, improved access to drugs and vaccines has enhanced infection control, using, for example, pre-emptive vaccination whilst a more judicious choice of infection control approaches (such as quarantine and travel restrictions) can be matched appropriately to specific situations. 'Microbial forensics' can be used to help establish attribution if a malevolent deployment is suspected.

New tools have improved the medical management of disease outbreaks, including intentional releases. Context-specific guidance to optimise response preparedness, for example for the use of anti-microbial drugs and decontamination options is now available. Superior protective equipment is now available, reducing the burden placed on responders and allowing them to work for longer, increasing the efficiency of a response. A wider range of decontaminants, and the optimisation of approaches for developing and using them, have reduced environmental transmission risks and released contaminated sites more quickly.

3.5.1 Improving community buy-in for responders

A response to an outbreak will only be effective if the communities involved are prepared to work with responders. Advances from anthropological studies have provided for significant progress in identifying and developing best practice for obtaining community buy-in for responding to disease outbreaks. Such buy-in is critical to speed the adoption of response and control measures,[459, 460] for ensuring that such measures are comprehensively applied[460] and to improve epidemiology and disease tracking efforts.[461]

Lessons learned from recent disease outbreaks included active engagement by anthropologists to:

- help communities adopt proven interventions;[459]
- scale up the adoption of these measures;[459]
- overcome cultural barriers from within communities;[459]
- overcome wariness of engaging with responders;[459]
- adapt measures to conform with traditional beliefs;[459]
- listening to the community and integrating their views into response efforts;[461]
- providing psychological assistance to families and people who have come into contact with the disease.[461]

3.5.2 Medical management and infection control (including quarantine)

Research since the Seventh Review Conference noted the most important driver in preventing pandemics is the breakdown or lack of public health infrastructure.[462] There have been indicators of increasing capacity for the prevention and control of certain infections, such as pandemic influenza.[463]

Technical developments discussed in the previous section of this report have had significant implications for medical management and infection control. Increased access to drugs, such as antivirals, has offered improved infection control options.[464] Pre-emptive vaccination in cases like pandemic influenza, has been demonstrated to be as effective at preventing death, and more cost effective, than reactive vaccination.[465]

New modelling tools have been developed for medical management of disease outbreaks, including intentional releases, including:

- modelling tools for pathogen movements and concentrations in indoor environments;[466]
- approaches for assessing the cost effectiveness of different interventions;[464]
- approaches for the eradication of key disease vectors, or the elimination of their ability to transmit the disease;[304]

- improving hospital designs to limit the transmission of pathogens whilst reducing energy usage;[467] and
- modelling disease transmission with different levels of community buy-in.[468]

These tools have allowed the development of context-specific guidance to optimise response preparedness, for example on the use of antibiotics,[466] approaches for evaluation and decontamination[466, 469] and assisted in the optimisation of response preparedness.[464]

Scientific research has been conducted to provide evidence as to the utility of some infection control measures, for example determining that patients wearing surgical masks do reduce aerosols responsible for viral transmission in influenza,[470] and the use of PPE in preventing the transmission of Ebola in health care settings.[471]

Traditional approaches to infection control, such as quarantine and travel restrictions, have continued to be used. Research on the use of quarantine as a disease control measure has highlighted political, ethical, and socioeconomic issues and stressed the importance of finding a careful balance between public interest and individual rights.[472] The value of social distancing in combating disease outbreaks like pandemic influenza has been demonstrated but has also been demonstrated to be a major contributor to the costs associated with low severity infections.[464] Domestic travel restrictions have also been demonstrated to limit transmission of pathogens, shortening the outbreak and enabling efforts to be focused on affected areas.[468]

3.5.3 Protective equipment

There have also been advances in protective equipment reducing the burden placed on responders and allowing them to work for longer, improving the efficiency of a response. Convergence of different scientific disciplines has led to improvements in protective equipment, in particular systems which enhance protection but with reduced physiological burden for the wearer, and which are less cumbersome. There are also research efforts to develop self-decontaminating protective clothing, e.g. with the incorporation of enzymes and/or catalysts'.[473] Advances in nanomaterials are also being applied to the filters and absorbents used in mask canisters and in 'protective clothing which may have a lower burden for the wearer than current systems'.[473]

3.5.4 Decontamination

A wider range of decontaminants, and approaches for developing them, have helped to reduce environmental transmission risks and release contaminated sites more quickly. Novel developments in nanotechnology have provided opportunities to improve existing decontaminants.[156, 474, 475] Advances in rational enzyme design provide new avenues for developing decontaminants.[475]

Research has optimised the use of decontaminants. There has been progress in assessing the efficacy of different decontaminants, for example sporicides[476, 477] and aerosol decontaminants.[478] Such assessments have also examined the impact of other factors, such as the type of material to be decontaminated,[476, 477, 478] and the structural design of the space to be decontaminated.[479] The impact on the type of agent used on decontamination efforts has also been explored, for example with *Bacillus anthracis*,[476] and *Brucella suis*.[477]

Studies have also been conducted to assess the potential for further aerosolization and spread of agents from decontamination efforts.[478]

4

Advances that reduce risks relevant to the BWC

There have been a number of developments that reduce overall risks relevant to the BWC. These include:

- Greater ability to identify indicators of prohibited activities (relevant to **Article I**). For example: increased opportunities for off-site sampling and bioforensic investigations derived from the persistence of genetic fragments throughout standard autoclaving techniques;[197] and improved tools to mark and identify synthetic biological parts, devices and products;[481]
- Barriers to acquiring biological agents for activities prohibited by the BWC in a manner that does not restrict their use for permitted purposes (relevant to **Articles III, IV and X**). For example, many gene synthesis companies have signed up to industry-led initiatives to screen customers and orders to prevent the use of their services in activities prohibited by the BWC;[480]
- Functional alternatives to capabilities which might be used for prohibited activities, that can be used for permitted purposes (relevant to **Articles III and X**), for example a reduced need for high containment facilities, facilities for culturing or holding live pathogens, and individuals skilled and experienced in culturing live pathogens: (a) for diagnostics, resulting from the development of metagenomics approaches for identifying pathogens from environmental samples without needing to culture them;[200] and (b) in vaccine production, resulting from the use of synthetic nanoparticles or virus-like particles in vaccine production and other developments.[365, 482]
- Alternatives to agents which might be used for prohibited activities without impacting their use for permitted purposes (relevant to **Articles III and X**) for example: the use of genome editing techniques to remove Ricin toxin production from castor plants;[483] and Novel biocontainment approaches designed to reduce the risk of gain-of-function research by negating the pathogenicity of an agent;[484]
- Progress in identifying activities with potential biosecurity concerns (relevant to **Articles III and IV**), for example, progress in developing risk-based, rather than taxonomic-based methodologies, for example, as part of the safety screening process of the International Genetically Engineered Machines competition.[485]

5

Developments in science and technology posing future risks for the BWC

The same innovations that have led to many of the positive benefits, have also facilitated prohibited activities relevant to every step of a biological weapons programme. This has resulted in the barriers to acquiring and using a biological weapon having been conspicuously eroded since the Seventh Review Conference. This has significant implications for Articles I, III and IV of the BWC.

Many of these developments are at the leading edge of current capabilities. They are expensive and complicated to acquire and deploy successfully. Making use of them for prohibited purposes would probably require the resources of a state. This situation may change in the future, reinforcing the need for on-going efforts to review relevant developments in science and technology.

This section provides a review of S&T developments with implications for any part of the process of making, producing and delivering a biological or toxin weapon can be considered as a potential biosecurity risk factor. The following sections consider each of the different stages of such a process:

- Developing a biological agent
- Producing and stockpiling a biological agent
- Dispersal and delivery of a biological agent

5.1 Developing a biological agent

Since the Seventh Review Conference there has been headway in:

- **Acquiring agents from nature**; including an expanded range of possible agents and locations in which they are to be found, as well as tools for characterising previously 'unculturable' microbes;
- **Synthesising existing agents**; non-specialists can now compile gene 'cassettes' coding for, for example, virulence factors and 'reboot' some viruses; pathogens responsible for historical epidemics can now be synthesised or reactivated; and many small peptides, bioregulators and toxins can now be produced by chemical synthesis;
- **Designing and synthesising novel agents**, this is now easier through using genome engineering platforms, 'cloud-labs', 'biofabs', and more sophisticated tools, and the availability of standards for designing, manipulating and compiling microbes, their parts, and proteins. This allows novel pathogens to be produced.

Neurobiology has also seen an exponential increase of output, improving our understanding of neural network responses associated with behaviours, such as anger and aggression, and physiological conditions such as addiction, fear and narcolepsy. Neural networks can now be manipulated to induce some of these states and work has begun to translate these findings to non-human primate models.

There is potential to develop other novel agents, including those produced using CRISPR/CAS9-mediated 'gene drives', 'gene silencing' technologies, proteins, or nanoparticles. Some of these types of constructs have already been inserted into vectors and demonstrated to produce effects in a host when administered by inhalation. The potential to target the microbiome to cause or exacerbate a disease state has also been highlighted.

Virulence and other biological features of pathogens are now more easily optimised for use in biological weapons and some 'enhanced pathogens' have been produced. This has been made possible by improvements in:

- Identification and characterization of the genetic components and key structural elements controlling pathogenicity, transmissibility, host range, antimicrobial defences, drug resistance, as well as in the mechanisms through which pathogens avoid the host's immune system;

- Applying an improved understanding of immunopathology to model the immune responses in a host;
- Using coatings and shells to confer environmental stability; and
- Developing tools for identifying and integrating desirable factors into biological agents.

Modern genome 'editing' technologies, such as CRISPR/CAS-9 do not leave 'fingerprints' indicating that that organisms has been altered. This could conceal attempts to enhance the organism's effectiveness, hampering forensic investigations and complicating the differentiation between unusual and unnatural disease events (see Box 4).

Box 4: An example of a novel biological agent to target plant and animal populations

The development of CRISPR-based 'gene drives' could enable individual laboratories to unilaterally alter the traits of wild populations and ecosystems without regard for national borders. Hundreds if not thousands of laboratories will have this capability within a few years. In principle, alterations can be undone by subsequent gene drive countermeasures, but must first be detected by environmental monitoring of at-risk species. This clearly requires detailed knowledge of whether those species can be affected.

There are currently only a handful of laboratories working in the CRISPR-gene drive field. Representatives of these groups as well as those in related areas have already called for transparency and safeguards to prevent accidental releases. Researchers in the field are now calling for all CRISPR gene drive research to publicly disclose experimental designs and safeguards against accidental release in advance of experiments. Transparency could ensure compliance with the BWC, accelerate the science by encouraging international collaborations, and promote early deliberations and community guidance of potential applications in public health, sustainable agriculture, and ecological conservation.

Technological advances have made it easier to access and characterise biological agents. An increased understanding of the properties of natural agents, such as pathogenicity and transmissibility, together with new tools enabling the design and redesign of biological agents, means that novel pathogens and toxins with enhanced properties can be generated.

5.1.1 Obtaining agents from nature

Access and characterization of a broad range of potentially harmful natural agents has become easier, faster and more accurate.

- The potential to develop a wider range of biological agents into weapons has been highlighted, for example, one report noted the role of fungal diseases in significant plant epidemics as well as characteristics desirable in a biological weapon's agent, such as environmental stability.[79]
- The identification of novel biological agents present in mixed environmental samples, including pathogens, is much faster, reliable and more accurate using recent metabolomics techniques.[190]
- The characterization of pathogens that have been traditionally unculturable has become possible with synthetic genomics approaches.[7]
- The number of locations from which it is possible to isolate agent has increased as our knowledge of the geographic distribution of certain pathogens has expanded, for example, discovering a Europe filovirus, a viral species previously only thought to exist naturally in sub-Saharan Africa.[242]

5.1.2 Synthesizing an existing agent

Since the Seventh Review Conference, it is possible to synthesize a wider range of pathogens and toxins, and DNA synthesis has become a lot more accessible and affordable.

- The tools and technology available for synthesizing agents have improved and become more accessible. For example, a desktop gene synthesizer is now commercially available.[486] The potential for non-specialists to use DNA synthesis to acquire pathogens has been demonstrated since the Seventh Review Conference, with two information technology professionals compiling and rebooting a virus from commercially-produced gene cassettes.[487]

- A wider range of strains of pathogens can be generated using DNA synthesis:
  - Pathogens responsible for major historical epidemics, including extinct ancestral viruses, can be generated using sequence data and attendant analysis;[244, 246, 385]
  - Agents difficult or impossible to culture from the wild,[201] organisms such as plasmodium[201] and highly pathogenic viruses such as Schmallenberg Virus[44] have been rescued and/or manipulated.
  - The synthetic design of yeast chromosomes[488] has progressed, and could lead to further developments in the manipulation of eukaryotic genomes.
- The range of small peptides, bioregulators and toxins it is possible to produce has expanded due to developments in chemical synthesis.[489, 490]

5.1.3 Adding functions to existing agents – Pathogenicity

The understanding of pathogenicity, its genetic and molecular basis, mechanisms and intensity modulation, have progressed. This is linked with the development of better tools in this domain.

- Genetic components conferring pathogenicity have been identified and characterised,[105] in particular in:
  - lethal bacterial strains, including *Escherichia coli* (O104:H4),[19] methicillin-resistant *Staphylococcus aureus*,[22] *Bacillus anthracis*[491] and *Mycobacterium tuberculosis*;[22]
  - influenza viruses, including influenza A H1N1,[21] H5N1,[21] and H7N9;[23, 24]
  - enteroviruses type 71;[21]
  - Dengue fever virus;[21]
  - an unculturable bat virus;[7]
  - wheat yellow rust.[27]
- Key structural elements affecting pathogenicity have been mapped and characterised, for example:
  - infection-specific structures in rice blast;[30]
  - structural changes in the HA and NA regions of human and bat influenza viruses.[7]
- Pathogens with increased virulence have been produced. Examples since the Seventh Review Conference include:
  - the evolution of viruses with increased virulence, using serial passaging in animals, including Schmallenberg Virus in a mouse model,[44] an engineered reassortant swine influenza virus in pigs;[9] Ebola virus in guinea pigs[13] and influenza H7N1 in ferrets as a preferred surrogate host for humans.[6]
  - the creation of a novel virus that is functionally similar to the causative agent of the 1918 pandemic, but with increased pathogenicity, using recombination of influenza viruses;[8]
- There has been progress in identifying ways in which pathogens cause damage by manipulating their host, for example, modulating the damage caused by cytokine storms and opportunities for conferring these abilities to other pathogens.[93]
- Our understanding of how pathogenicity factors spread in the wild, such as by horizontal gene transfer, has improved;[38]
- More sophisticated tools have been developed for identifying factors associated with pathogenicity and how they are integrated into agents, for example:
  - computational approaches and software tools for the identification of pathogenicity islands and virulence factors;[22, 39, 40]
  - synthetic biology approaches;[16, 41, 65]
  - bioinformatics tools for manipulating pathogenicity data;[41]
  - systems biology and understanding the impacts of systems modulation on disease;[41, 492]
  - whole genome directed evolution;[16]
  - methodological approaches for testing the clinical and public health significance of microbe-disease associations;[42]
  - replacing wild-type regulatory networks controlling biological function with better characterized engineered versions;[492]
  - improved tools for identifying biological parts which can be used in designing and building engineered systems and functions;[492]
  - approaches for predicting the virulence of a pathogen from its sequence information;[22]
  - the creation of a centralised repository for pathogenicity islands and resistance islands;[43]
  - model systems that recapitulate *in vivo* viral life cycles.[18]

5.1.4 Adding functions to existing agents – Circumventing host immunity

The understanding of host-pathogen relationships has improved, including how pathogens can avoid or modulate the immune responses in their hosts. A broader range of pathogens have been studied. The development of better tools has supported these advances.

- The understanding of host-pathogen relationships enabling the modulation of immune responses in the host has improved. Examples include:
  - reducing or removing cytokine storms;[93]
  - the inflammatory mechanism in gut bacteria, including the horizontal gene transfer of key factors;[38]
  - the identification of a novel neutrophil-based immune response to viruses;[97]
  - the use of non-structural proteins to interfere with a host's epigenome and make cells more susceptible to viruses;[84]
  - modulation of host proteins which inhibit viral replication;[85]
  - infection kinetics and the impact of the physical location of pathogens in a host on the speed of infections;[88]
  - the use of natural (e.g. polysaccharide) or manmade (e.g. polyelectrolyte based nano-thin polymer) coatings to isolate cells from the host's immune system;[89, 100]
  - gene silencing in a virus to enable a dormant state in the host's neuron's, dramatically increasing the persistence and re-emergence of infections;[22]
  - degradation of the functional capacity of host macrophages, enabling pathogens to survive inside them.[87]
- The understanding of the mechanisms through which pathogens avoid the host's immune system has progressed. Examples include:
  - proteins used by pathogens to suppress immunity in plants;[102]
  - mechanisms for negating innate immunity;[22, 35, 47, 86, 87, 89, 91]
  - mechanism for negating vaccine-acquired adaptive immunity;[22, 89]
  - modulation of the production of viral proteins used to block interferon production in the host;[44]
  - a viral mechanism using RNA motif's to avoid a host's immune system;[103]
  - chemical modification of the pathogen's genetic material to void detection;[87]
  - the production of proteins by a pathogen to interfere with the host's ability to trigger an immune response,[87] including type III secretion processes; antigenic variation;[87]
  - the use of TAM receptors to inhibit innate immune response;[87]
  - interference with receptor proteins on host immune cells;[87]
  - broadly reactive antibody-binding proteins used to block antibody-antigen binding.[87]
- Host-pathogen relationships have been studied for an expanding range of pathogens. Examples include:
  - Enterobacteriacea,[38] *Yersinia pestis*,[35, 47, 87, 89, 90] *Salmonella enterica*,[87] *Brucella* sp.,[87] *Mycoplasma* sp.;[87] *E.coli*,[87] *Staphylococcus aureus*;[92]
  - influenza viruses,[84, 85, 86, 87] Schmallenberg Virus,[44] *flaviviruses*,[91] Dengue virus,[87] SARs coronavirus,[87] West Nile virus,[87] varicella zoster virus,[22] vaccinia virus.[87]
- More sophisticated tools for modulating host-pathogen interactions have been developed, for example:
  - synthetic biology approaches;[41, 65]
  - systems biology and understanding the impacts of systems modulation on disease;[41, 492]
  - replacing wild-type regulatory networks controlling biological function with better characterized engineered versions;[492]
  - improved tools for identifying biological parts which can be used in designing and building engineered systems and functions;[492]
  - model systems that recapitulate *in vivo* viral life cycles.[18]

5.1.5 Adding functions to existing agents – Transmissibility and host range

The identification and characterization of genetic and structural components conferring transmissibility and host range has progressed. Pathogens with altered transmissibility or host range have been isolated or produced. The development of better tools has supported these advances.

- There has been progress in the identification and characterization of genetic components conferring transmissibility and host range.[105] Examples include:
  - specific mutations which confer aerosol transmission in mammals, in influenza viruses including in H5N1,[1, 2, 3, 4, 5] H7N1,[6] and H1N1;[8, 9]
  - mutations in HA region of H5N1 influenza virus that improve affinity for humanlike airway receptors;[5]
  - sequencing of a H7N9 influenza virus adapted for efficient growth in human lung tissue;[11]
  - mutations in H7N9 influenza virus conferring antiviral resistance with no impact on transmissibility;[12]
  - a specific mutation in H7N9 influenza that confers an ability to bind to both human and avian receptors,[10] and specific mutations in H1N1 influenza virus mutations associated with host range[13]

- Pathogens with increased transmissibility have been produced or isolated. Examples include:
  - a H5N1 and H1N1 recombinant influenza viruses capable of aerosol transmission in ferrets;[1, 5]
  - the use of site-directed mutagenesis and serial passaging in ferrets to confer airborne transmission on the H5N1 and H7N1 influenza viruses;[2, 6]
  - a H7N9 influenza virus adapted for efficient growth in human lung tissue was isolated;[11]
  - a recombinant human and bat influenza virus;[7]
  - and the use of serial passaging of an engineered reassorted H1N1 influenza virus in pigs.[9]
- Our understanding of how key structural elements affect host range has progressed, for example, how the structure of HA regions affect H5 influenza viruses ability to bind to avian and human receptors[14] and similar insights for H7N9 influenza viruses.[10]
- Pathogens with an altered host range have been produced. Examples since the Seventh Review Conference include: enteroviruses type 71, H1N1 influenza virus, H5N1 influenza virus, and Dengue fever virus.[4]
- More sophisticated tools for identifying factors associated with transmissibility and host range and integrating them into agents have been developed, for example:
  - tools to study genetic evolution of viruses and monitor for mutations known to confer altered transmissibility;[3]
  - synthetic biology approaches; [16, 41, 65]
  - systems biology and understanding the impacts of systems modulation on disease;[29, 41, 492]
  - replacing wild-type regulatory networks controlling biological function with better characterized engineered versions;[492]
  - improved tools for identifying biological parts which can be used in designing and building engineered systems and functions;[492]
  - model systems that recapitulate *in vivo* viral life cycles.[18]

5.1.6 Adding functions to existing agents – Antimicrobial and drug resistance

Advances in tools have allowed the identification and characterisation of genetic and structural elements that affect antimicrobial functions and drug resistance. Pathogens with increased antimicrobial properties and drug resistance have been produced. In addition, we have a better understanding of mechanisms at the community level explaining how pathogens become resistant to antimicrobial therapy.

- An increasing number of specific mutations associated with antimicrobial functions and drug resistance have been identified and characterised.[213, 493] Examples include specific mutations conferring:
  - vancomycin resistance in *Staphylococcus aureus*[426] and enterococcal bacteria;[424]
  - Carbapenem resistance in *E.coli*,[421] in *Enterobacteriaceae*,[419] in Pseudomonas aeruginosa[494] and in Acinetobacter sp.;[422]
  - neuraminidase-inhibitor resistance or reduced sensitivity in influenza viruses,[423] including in H7N9 influenza virus without loss of *in vivo* virulence or transmissibility;[12]
  - methicillin resistance in *Staphylococcus aureus*;[245]
  - artemisinin resistance in P.falciparum;[418]
  - resistance to oseltamivir in influenza A viruses;[427]
- Our understanding of how key structural elements affect antimicrobial functions and drug resistance has progressed. Examples include:
  - differences of neuraminidase structure on the efficacy of inhibitors;[423]
  - how the structure of the M2 ion channel impacts the antiviral drugs amantadine and rimantadine;[429, 430]
  - and the mechanism for artemisinin resistance in P.falciparum.[432]
- There have been advances in understanding mechanisms at the community level taken by pathogens to become resistant to antimicrobial therapy, for example the use of dormant persister cells in Mycobacteria,[434] *E.coli*,[436, 494] *Burkholderia pseudomallei*[438] and associated mechanisms.[435, 495]
- Pathogens with increased antimicrobial properties and drug resistance have been produced. Examples include: Salmonella;[440] *Bacillus anthracis*;[440] *E.coli*;[440] *Vibrio cholerae*;[440] and *P. falciparum*.[439]
- More sophisticated tools for identifying factors associated with antimicrobial function and drug resistance and integrating them into agents have been developed, for example:
  - improved understanding of how antibiotic resistance spreads in the wild, such as by horizontal gene transfer;[38]
  - synthetic biology approaches;[41, 65]
  - bioinformatics tools for manipulating drug resistance data;[41]
  - systems biology and understanding the impacts of systems modulation on disease;[41, 492]

- CRISPR/CAS9 genome editing tools;[439]
- microfluidics;[495]
- reporter genes;[495]
- replacing wild-type regulatory networks controlling biological function with better characterized engineered versions;[492]
- improved tools for identifying biological parts which can be used in designing and building engineered systems and functions;[492]
- transcriptomics;[432]
- the creation of a centralised repository for pathogenicity islands and resistance islands;[43]
- model systems that recapitulate *in vivo* viral life cycles.[18]

5.1.7 Adding functions to existing agents – Environmental stability

The understanding of how structure affects the environmental stability of biological agents has increased. More environmentally stable pathogens were produced, and stability has also been artificially conferred using coatings and shells. Advanced tools have been developed:

- Our understanding of how structure affects environmental stability has progressed, for example, how electrostatic repulsion inside the capsid of Foot-and-Mouth Disease viruses is responsible for its thermolability.[318]
- Coatings and shells have been developed to confer environmental stability, examples include:
  - environmentally-responsive smart delivery platforms, such as nanoparticles;[376]
  - artificial spores protecting agents against osmotic pressure, shear force, heat, UV radiation, and lytic enzymes;[157]
  - polyelectrolyte based nano-thin polymer coatings.[100]
- Pathogens with increased environmental stability have been produced, examples include lactic acid bacilli, *Francisella tularensis*,and Salmonella sp..[496]
- More sophisticated tools for identifying factors associated with environmental stability and integrating them into agents have been developed, for example:
  - improved understanding of how antibiotic resistance spreads in the wild, such as by horizontal gene transfer;[38]
  - synthetic biology approaches;[41, 65]
  - bioinformatics tools for manipulating environmental stability data;[490]
  - systems biology and understanding the impacts of systems modulation on disease;[41, 492]
  - computation tools for the rational design of stabilised enzymes;[269]
  - replacing wild-type regulatory networks controlling biological function with better characterized engineered versions;[492]
  - as well as improved tools for identifying biological parts which can be used in designing and building engineered systems and functions.[492]

5.1.8 Designing a novel agent

Advances in genome engineering technologies, nanoparticles, neurobiology, and protein science could be used to design or redesign biological agents. Genome engineering technologies have significantly advanced, in particular new gene editing tools (e.g. CRISPR/CAS9) could potentially lead to the development of novel pathogens and/or alter entire populations.

- There has been significant progress in being able to design and build an agent from scratch.[493, 497] Examples include:
  - progress in the rational redesign and synthesis of the yeast genome;[498, 499]
  - advances in controlling and measuring cell-environmental interactions in vitro;[273]
  - designed organisms to produce lysine, xanthan and acarbose.[500]
- The potential to target the microbiome to cause or compound a disease state has been noted.[501] Probiotics have been engineered to detect and record environmental signals in the gut as diagnostics, but potentially they might also be engineered to cause harm.[144, 163]
- Genome engineering technologies facilitating the comprehensive redesign of agents have significantly advanced. Examples include:
  - preassembled zinc-finger arrays for rapid construction of Zinc Finger Nucleases;[502]
  - increased understanding of optimized transcription activator– like effectors;[503]
  - a mechanism for genome-wide codon replacement enabling the precise manipulation of chromosomes *in vivo*;[504]
  - improvements in designed transcription activator-like effector nucleases (TALENs);[500, 505]
  - CRISPR/CAS9 genome engineering tools,[506] including for viral delivery,[384] editing higher primates;[500]
  - associated computational design tools.[507]

- Novel pathogens have been produced, for example:
  - an engineered microbe to sense and eradicate Pseudomonas aeruginosa[284]
  - a highly virulent, recombinant H1N1 influenza virus;[8]
  - warnings have been made on the potential to develop other novel pathogens, for example using CRISPR/CAS9 mediated gene drives to 'alter populations of agricultural plants or livestock by actors intent on doing harm'.[508, 509] CRISPR/CAS9 elements (without gene drives) have been inserted into viral vectors and used to edit a target genome via inhalation in a mouse model.[384]
- Particular progress has been reported in understanding the neurobiology of physiological conditions such as narcolepsy, addiction and fear.[114, 116] Publications in the field have expanded exponentially since the Seventh Review Conference:[510]
  - Novel techniques have allowed neurological responses to be studied in real time and have helped identify neurological pathways associated with addiction and fear, offering potential new targets for agents.[115, 511]
  - Progress has also been made in modulating behaviours.[116] For example application of advances in understandings have enabled researchers to initiate sleep in mice by triggering certain neurons.[118]
  - They have also linked responses in neuron networks to psychological conditions, for example characterizing neurological states that provoke anger or aggression.[119]
  - Recent work has also translated tools for characterizing and manipulating these states into non-human primate models.[510]
- Novel agents based upon genetic material have entered into clinical trials, including those based on siRNAs.[291]
- Novel protein-based agents have also been identified.[156, 512] Examples include:
  - small molecule drugs that could be used to initiate dangerous hypersensitivity reactions;[104]
  - the identification of a range of proteins critical for the survival of *E.coli*, which is sometimes used as a chassis for biosynthesis;[276]
  - the misuse of neuropharmacology and neuropeptides;[257, 513]
  - misuse of bioregulators;[156, 257, 514]
  - a protein able to bind with high affinity to all types of human and nonhuman immunoglobulin G' antibodies, blocking antibody-antigen union;[125]
  - a computationally designed protein to stimulate cells infected with the Epstein Barr virus to kill themselves;[260]
  - chemically modified, metabolically-resistant analogues of peptides with increased potency and toxicity.[514]
- Novel agents based on nanoparticles that can penetrate cells have also been developed.[500]
- There have also been advances in delivering these agents, for example with environmental responsive,[374] or remotely activated[414] nanoparticles.
- More sophisticated tools for designing, manipulating and compiling agents have been developed, for example:
  - synthetic biology approaches;[284, 364, 497, 501, 515, 516, 517, 518]
  - improved computational enzyme design;[269, 518, 519, 520]
  - bioinformatics approaches for extracting data from past publications;[268]
  - rational design principles for virus-like particles;[309]
  - experimental-computational technology for inferring network models that predict the response of cells to perturbations;[309]
  - improved understanding and engineering of regulatory elements of synthetic metabolic pathways;[492]
  - directed evolution;[518]
  - computational methods,[259, 269, 519, 520, 522]
  - software tools and programming languages that compress the time required for converting design of a recombinant vector to its delivery;[500]
  - larger scale, higher fidelity production platforms for genetic material;[282]
  - as well as an integrated design suite for manipulating yeast, including software tools 'for the prediction of biochemical pathways, molecular biology methods for assembly of DNA parts into pathways, and for introducing the pathways into the host, and finally approaches for optimizing performance of the introduced pathways'.[521]
  - Cloud-based laboratories offering fully remote services and facilities offering services designed to support each of these steps have begun to appear since the Seventh Review Conference.[278]

- More sophisticated tools for designing, manipulating and producing proteins have been developed, for example:
  - general design tools for proteins;[276, 364]
  - design tools for proteins to bind with surface structures;[259]
  - data-driven predictions of drug effects and interactions;[261]
  - high throughput approaches to toxicological screening;[257]
  - improved computational enzyme design;[269, 518, 519, 520]
  - and computation design of proteins to inhibit enzyme function.[522]

5.1.9 Toxins

The genetic components and mechanisms of action of toxins are increasingly well characterized and their characteristics can be manipulated. More sophisticated tools for researching and manipulating toxins have been developed. Biosynthesis metabolic pathways can now be engineered providing alternative production routes for toxins. This could be particularly important in the case of those toxins that are awkward to extract in large quantities from natural sources.

Advances in tools have made it easier to produce toxins on a large-scale, which has commercial applications but also means it complicates efforts to prevent the use of toxins as bioweapons.

Shortcomings in the quality assurance of current detection capacities have revealed vulnerability in our ability to deter or mitigate the use of toxins as weapons. Novel agents, or those with altered or enhanced characteristics have been identified or produced since the Seventh Review Conference. There is also a need for better standards in detecting toxins to be enforced.

- More genetic components associated with toxins have been identified and characterized, examples include:
  - 743 mutations over 12 human genes important for intoxication by four different cytolethal distending toxins;[50]
  - determining human genetic variation determines sensitivity to the anthrax toxin;[51]
  - publication of sequence and transcription data for king cobra venom.[52]
- Our understanding of mechanisms of action associated with toxins has improved,[156, 493] examples include:
  - increased understandings in the cellular mode of action of Ricin toxin;[55]
  - characterization of the metabolic pathway to produce Saxitoxin;[57]
  - modulation of binding immunoglobulin protein to increase cytotoxicity;[58]
  - characterization of structural interactions between Botulinum toxin and host cells.[63]
- Novel toxins, or those with altered or enhanced characteristics have been identified or produced, examples include:
  - an anthrax toxin with altered receptor specificity;[66]
  - a recombinant Botulinum type A – tetanus toxin;[69]
  - a novel form of Botulinum toxin.[67]
- The existence of relatively large-scale industrial and cosmetic uses of toxins, such as Botulinum toxin, complicates efforts to prevent its use as a weapon.[350]
- Use of metabolic pathway engineering and biosynthesis provides alternative production routes that could overcome challenges of harvesting sufficient toxins from nature.[65, 241, 350, 523]
- Current shortcoming in ensuring standards in detecting toxins highlighted vulnerability in deterring or mitigating their use as weapons.[78]
- More sophisticated tools for researching and manipulating toxins have been developed, for example:
  - synthetic biology approaches;[41, 523]
  - bioinformatics tools for manipulating environmental stability data;[41]
  - systems biology and understanding the impacts of systems modulation on disease;[41]
  - metabolic pathway engineering;[64]
  - computational design tools leveraging advances in understanding in structural-functional relationships.[66, 524]

5.2 Producing and stockpiling biological agents

Since the Seventh Review Conference, there has been notable progress or changes in:

- **Concealing prohibited activities.** Changes to production signatures and a shift towards the use of multiple smaller reactors compromises efforts to identify sites of biological weapons production;

- **Industrialising biological production processes.** Less space and time are now required for scale up, narrowing windows for interdiction. The process can also be simplified using new technologies, though at significant cost;
- **Producing biological agents.** The increased use of biosynthesis and bio-based production, scaffolds, and 'biopharming' accelerates the speed and yield. This also applies to vaccine production;
- **Switching production from permitted to prohibited activities.** The use of single-use, disposable and modular production equipment offers possibilities for faster technological breakout;
- **Acquiring relevant equipment.** Critical laboratory materials such as reaction vessels (including those currently covered by control lists) can now be fabricated using 3-D printing technology, reducing the costs and potentially lowering barriers to prohibited activities. Once again, this complicates efforts to enforce non-proliferation measures.
- **Distributed production.** The decoupling of design and manufacture has led to the growth of stand-alone fabrication and production facilities. Whilst limited in number of geographic distribution at the moment at the moment, the potential for the growth of such facilities and their impact in changing the footprint of prohibited activities might warrant closer attention over the coming years;
- **Outsourcing biological production.** Multipurpose biological production facilities suitable for varying-scale production of biological agents as well as for the synthesis of genetic material and other synthetic genomics techniques are now commonplace. The existence of many 'virtual' biotech companies demonstrates the potential in this space;
- **Storing biological agents.** Increasing the environmental stability of biologics, together with the use of other approaches removes the requirements for cold-chain storage and its associated infrastructure. Improvements in production techniques have reduced the need for 'stockpiling' whilst the proliferation of freeze-drying capabilities enables this should it prove desirable.

5.2.1 Changing footprint of production

Production signatures have changed and made it easier to potentially conceal prohibited activities. Both the equipment size and timeframe for production have been significantly compressed.

- Developments in scale-up and production technologies have changed production signatures, potentially helping to conceal prohibited activities and reduce windows for interdiction. The space required for production has been drastically reduced, examples include:
  - the use of suspended cultures in vaccine production;[329, 330, 525]
  - the use of egg-free systems in vaccine production;[331]
  - industrial use of microreactors for chemicals and small biological;[526]
  - baculovirus/insect cell expression systems to produce recombinant proteins and vaccines;[329, 333]
  - and the potential to use yeast-based systems for opinoid production.[334, 335]
- The physical size of production equipment has been drastically reduced, examples include:
  - the use of suspended cultures in vaccine production[330, 525]
  - industrial use of microreactors for chemicals and small biological.[526]
- There have been changes in the acquisition of relevant equipment, for example, through:
  - the potential to 3D print reaction vessels (including those currently covered by control lists);[527]
  - synthetic biology approaches have also been used to produce plastics for use in 3D printing machines, potentially altering the usual supply chains.[528]
- The timeframes needed to develop production processes have been compressed, for example, through the use of computational methods, software tools and programming languages.[527]

5.2.2 Industrial scale up

Scaling up has been simplified and made faster and cheaper in some cases.

- The time required to scale up processes has fallen in some cases. For example, increased automation and miniaturization of development processes has occurred since the Seventh Review Conference, in some cases process rated 'increased by magnitudes'.[529] Whilst these processes happen faster, they can still take years.[336]
- Certain costs associated with industrial scale up have fallen, for example, an estimated 90% reduction of the cost of purification by removing reliance on animal serums during production.[530]

- In other cases, improvements in efficacy and efficiency have increased costs, examples include:
  - more wide-spread use of cell culturing production, potentially increasing production costs by 2 – 3 fold;[530]
  - full automation and miniaturization of development processes requiring significant initial investments of hundreds of millions of dollars.[529]
- Certain scale-up processes have been simplified, examples include:
  - the use of directed evolution to remove reliance on animal serum thereby removing a challenging purification step that has caused regulatory concern;[530]
  - improvements in the media used in separation processes, which can account for 60% of downstream processing costs;[345]
  - the improved engineering of strains prior to scale up, especially for replicability and strain stability;[338, 529]
  - and use of virus-like particles instead of viruses, enabling 50 fold increased yield in some cases.[321]
- Purification steps have been improved or simplified and there has been growing regulatory attention paid to ensuring viral removal or inactivation,[344] examples include:
  - the improved use of directed evolution;[530]
  - multimodal chromatography;[345]
  - increased automation and miniaturization of processes;[529]
  - the use of UV-C irradiation for viral inactivation;[346]
  - research to determine the comparative efficacy of different viral inactivation techniques.[347]

5.2.3 Microengineering and microfluidics

Progress in microengineering and microfluidics make it possible to experiment with small samples, thus reducing cost and increasing efficiency.

- There have been improvements in producing biological components for experimentation and production at the microscopic scale, examples include:
  - 'a chip-based method that creates uniformly sized vesicles in assembly-line fashion';[279]
  - the use of nanoparticles for partitioning bulk material.[531]
- The reaction vessels used for experimentation and production at the microscopic scale have been improved, for example, there have been advances in flow microreactors.[532]

5.2.4 Bio-based production and biosynthesis

Bio-based production and biosynthesis have become increasingly common methodologies. One 2012 review 'identified 68 products across seven sectors (including biofuels, chemicals, energy, food, materials, and medicine) being developed by companies in 10 countries'.[533] A subsequent report by the US National Academy of Sciences recognized the increasing industrialization of biology and the importance of biological-based processes for the advanced manufacturing of chemicals.[481]

- There have been improvements in the tools enabling the controlled design and production of biological materials, examples include:
  - the application of recombinant DNA technologies to the development of conjugate vaccines;[311]
  - transient gene expression in serum-free suspension-growing mammalian cells for the production empty viral capsids;[330]
  - increasing ranges of microbial chassis,[533] in particular for clinical applications;[274]
  - directed evolution of enzymes;[533, 534]
  - the use of TALENs genome editing platforms;[534]
  - increased miniaturization and automation of equipment;[534] and
  - progress in developing standardized biological parts and devices[349, 534, 535] and advances in controlling and measuring cell-environmental interactions in vitro, including strategies for high-throughput analysis.[273]
- There have been significant developments in efforts to build designer yeast since the Seventh Review Conference. Such a yeast might offer opportunities for enhanced chassis for bio-based production and biosynthesis. Recent developments have included:
  - the use of tags enabling the differentiation of synthetic components from wild type counterparts, especially the development of a real-time PCR assay for their detection;[353]
  - an *in vivo* method for assembling designed DNA fragments;[354]
  - a novel genetic assembly system for compiling expression pathways.[355]

- Bio-based production and biosynthesis approaches have been applied to, or enabled the production of, a wider range of materials and agents, examples include:
  - new secondary metabolites with novel therapeutic activities, such as complex polyketides, halogenated and alkaloids;[349]
  - opinoids;[352]
  - toxins;[350, 534, 536]
  - peptides;[533]
  - an expanded range of biochemical reactions;[64]
  - biohybrid materials for drug delivery;[349]
  - delivery devices for vaccines;[351]
  - the industrial scale production of a precursor for an anti-malarial drug;[338]
  - additional industrial scale production of ethanol;[533]
  - the industrial scale production of biofuels, anthranilic acids, terpenoids; [533]
  - the commercial production of lysine,[534] xantham,[534] acarbose,[534] algal betaine surfactant;[537]
  - the commercial production of co-stimulators of immune responses for use with vaccines;[351]
  - proof of principle studies have also demonstrated the ability of similar approaches to produce plastics for 3D printers.[528]
- Advances have also simplified the bulk production material.[364]

5.2.5 Scaffolds

Molecular scaffolds have been applied to increase the efficacy of production of various products, and can be used for industrial production.

- Scaffolds made of various materials have been used to increase the efficacy of production processes by controlling the spatio-temporal arrangement of components, examples include, DNA scaffolds and nanostructures;[356, 357]and proteins.[358]
- Scaffolds have been used to enhance the efficacy of the production of different products, examples include:
  - resveratrol;[356]
  - 1, 2 – propanediol;[356]
  - mevalonate;[356]
  - antigen-adjuvant complexes;[357]
  - discrete and complex chemicals.[357]
- Scaffolds have also 'been developed for use in bacteria and yeast used for industrial production'.[357]

5.2.6 Biopharming

Increasingly, genetically engineered plants and animals are used to produce pharmaceutical substances for use in humans or animals.

- Plants have been used to produce an increasing number of products, examples include:
  - a treatment for Gaucher's disease grown in carrot cells which received licensing approval;[359]
  - a vaccine candidate for H1N1;[360]
  - monoclonal antibodies to treat Ebola;[361, 362]
  - antibodies to bovine viral diarrhea virus;[363]
  - antigens for rabies, hepatitis B, measles, avian influenza, anthrax.[363]
- Seeds have been engineered to act as storage devices for biological products.[538]
- Animals have also been engineered to produce useful products, such as recombinant proteins and antigens for vaccine candidates against malaria expressed in the milk of transgenic goats. 'In the short term, the aim is to harvest and purify the protein from the milk for vaccine production and testing, but the long term vision is to deliver the vaccine orally in the milk'.[363]
- Improved tools enable the controlled design and production of modified plants and animals, examples including the use of TALENS genome editing tools;[539] and the development of standardized biological parts and devices.[535]

5.2.7 Outsourced production and modular facilities

New ways to outsource productions, and modular facilities, mean that there are more ways to undertake prohibited activities.

- Improvement in the synthesis of genetic material and other synthetic genomics techniques has enabled the outsourcing of biological products, for example vaccine candidates,[336, 337] allowing for decentralised, compartmentalised development and production approaches.
- There are multipurpose biological production facilities suitable for outsourcing production, for example a multipurpose fermentation facility was used for the industrial production of a precursor for an anti-malarial drug.[338]

- Proof of principle for the development and production of biotechnology-based products have also been realised, for example through the advent of virtual biotech companies which in some cases 'have no employees or laboratory space but outsource all the work to consultants'.[540]

5.2.8 Disposable, synthesized and repurposed equipment

The shift towards the use of single-use, disposable and modular production equipment reduces the production time, offering possibilities for faster technological breakout, as well as complicating efforts to detect prohibited activities. The use of multiple smaller reactors, rather than one large one, challenges traditional proliferation controls that tend to focus on large scale production capacity to reduce regulatory burdens. In addition, the development of equipment for use in resource limited settings reduces costs for prohibited activities.

- The prevalence of single-use equipment is growing:
  - one industry study 'estimates that more than 40% of integrated single-use systems are now used in GMP manufacturing'. The authors noted 'several large biotech companies employ fully disposable processing, including chromatography, for smaller, mid-titre, clinical trial manufacturing processes'.[343]
  - An April 2013 review highlighted single-use reactors made from advanced polymers was expanding and that 'a number of bioprocess analysts estimate that the entire single-use market has been growing at about 15 – 20% per year'.[541]
  - An August 2012 industry survey of 300 biomanufacturers 'indicated a clear preference' for single-use bioreactors. Two-thirds of respondents indicated that they expected to implement batch-fed single use bioreactors at the clinical scale (as opposed to just under a third who expected to use stainless steel batch–fed bioreactors). At the commercial scale, over half of respondents expected to still be using stainless steel (which is a significant reduction from the current market share).[542]
- Disposable equipment has been used, or is being developed for use, in the production of:
  - vaccines;[339, 341, 543]
  - monoclonal antibodies;[344]
  - recombinant proteins;[341]
  - stem cells and personalised medicine.[341]
- The range of processes for which disposable equipment is available has increased in number and complexity, examples include:
  - tangential flow filtration;[343]
  - mixing and perturbing materials;[343]
  - downstream processing equipment;[339, 543]
  - there have also been reports as to the potential to use 3D printing to produce complex reaction vessels, including those covered by control lists potentially further complicating export controls.[544]
- There has been increasing standardization of parts, making it easier to switch to disposable equipment including 'standardized, off-the-shelf assemblies for cell culture harvest and tangential flow filtration, disposable, aseptic, genderless, universal connectors, and non-proprietary films and materials of construction for its bags, connectors, and tubing'.[343]
- The operating volumes of single use equipment have also increased, for example 'working volumes for integrated single-use bioprocess containers, systems, and support equipment have steadily risen from sub-1,000 – L levels (50 – 500 L) to the 2,000 L range, with prototypes exceeding 5,000 L for some applications'.[343] An April 2014 review noted '17 manufacturers offer around 80 – 90 models of single-use bioreactors with working volumes ranging from as low as few millilitres to up to 2000L'.[341]
- There has also been a trend towards using multiple smaller reactors, rather than a single large batch production for two reasons:
  - 'end-users feel comfortable with "scaling out" through the use of multiple 500 L or 2,000 L systems rather than scaling up to 5,000 L and higher';[343]
  - 'targeted therapeutics and higher titres are causing the slow extinction of mega-processes. Those that remain increasingly turn to multiple 1,000 L or 2,000 L disposable bioreactors to mitigate risks associated with batch loss'.[343]

- Equipment has also been developed for use in extremely resource-limited settings, for example:
  - PCR diagnostics which can work in the complete absence of electricity.[250]
  - A paper-based microscope has been produced, including both lower and higher resolution versions costing $0.5 and $1 respectively. Disease specific versions have also been developed, for example for malaria.[187]
  - An alternative approach has been to repurpose equipment, for example, a commercial DVD drive can be converted into a laser scanning microscope that can analyse blood and perform cellular imaging with one-micrometre resolution.[188]

5.2.9 Modification of agents, freeze-drying and non-cold chain storage

Although production trends could negate the need for stockpiling agents or reduce barriers substantially, the modification of agents to increase their environmental stability, and the proliferation of freeze-drying capacity have increased access to stockpiling capabilities should it be necessary. In this area, knowledge that was once almost exclusively in the hands of offensive and defensive biological weapons programmes has been published and made widely available. In addition, seeds can be engineered to act as storage device for biological agents.

- Agents have been successfully modified to increase their environmental stability and to remove the requirements for cold-chain storage, thereby simplifying the stockpiling of biological agents.
- The mechanisms through which biological agents tolerate environmental stresses are better understood, for example in fungi,[545] and the identification of key properties in conferring tolerance to freeze drying.[546]
- Alternatives to live agents have been developed, negating the need for cold chains, for example the use of empty viral capsids.[317]
- A number of different approaches have been developed to increase the environmental stability of biological agents and, in some cases enable room temperature storage, examples include:
  - self-standing silk protein biomaterial matrices;[366, 367]
  - coating viral capsids with a mineral coat, or other materials;[368, 547]
  - engineering structural changes into viral capsids.[318, 547]
- Formulae for freeze-drying bacteria, and an assessment of their comparative efficacy have been published.[546]
- Improvements have also been made to drug delivery vehicles, such as nanoparticles, to make them more environmentally stable.[369]
- Seeds have been engineered to act as storage devices for biological products.[538]

5.2.10 Microencapsulation and smart particles

Drug delivery technologies have greatly progressed. In particular, the understanding, development and uses of nanoparticles has expanded.

- An increasingly sophisticated understanding of the structural-function relationship of nanoparticles has led to improvements in using them as drug delivery vehicles.[370, 405, 548]
- Drug delivery devices have improved. Some have translated into marketable products, and novel types of drug delivery carrier have been created:
  - A July 2011 review noted that they often fail to meet clinical expectations but progress in re-engineering natural carriers which are 'highly optimized for their specific functions *in vivo* and possess features that are often desired in drug delivery carriers'.[407]
  - A May 2012 review suggested that nanoparticle carriers were making 'it possible to increase the therapeutic index of many components'. The authors listed a limited number of nanocarriers that were already on the market or in phase III trials.[372]
  - An April 2014 review noted 'advanced drug delivery systems based on micelles, polymeric nanoparticles, and dendrimers' and that 'polymeric carbon nanotubes and many others demonstrate a broad variety of useful properties'.[408]

- The range of drug delivery platforms has increased, examples include:
  - the use of lipid-based liquid crystalline nanoparticles;[380]
  - magnetic core-shell nanoparticles for aerosol drug delivery;[381]
  - cubic phase nanoparticle for sustained release of ibuprofen;[382]
  - self-assembled liquid crystalline nanoparticles;[383]
  - nanoparticles controlled by ultraviolet light;[409]
  - a remotely activated protein-producing nanoparticle;[414]
  - aerosol optimised delivery nanoparticles;[376, 381]
  - environmentally-responsive nanocarriers;[376, 403, 415, 548]
  - biodegradable nanoparticles;[548]
  - delivery platforms intended to circumvent the immune system.[403, 541]
- Drug delivery devices have been developed with increased abilities to cross barriers, examples include:
  - enhanced tissue penetration;[409]
  - a post-facto undetectable nanoparticle carrier designed to deliver siRNA through the skin;[376, 410]
  - delivery across the blood-brain barrier;[411, 412, 549]
  - nanoparticles designed to penetrate cell membranes.[550]
- Drug delivery technologies have been developed to overcome specific challenges, examples include:
  - overcoming low oral absorption rates;[383]
  - overcoming rapid metabolization of drugs;[377, 383]
  - improved activity;[372, 376, 377, 413]
  - reduced toxicity;[372, 408, 549]
  - extended time circulating in the blood by reducing clearance by the liver.[551]
- Developments have also enabled the encapsulation or coating of biological agents to make them more environmentally stable, for example using nanobiological membranes[411] or polyelectrolyte based nano-thin polymer coatings.[100] An August 2013 review of artificial spores highlighted that they could be used to confer 'extensive cytoprotective capabilities that encompass exposure to osmotic pressure, shear force, heat, UV radiation, and lytic enzymes'.[157]
- Nanoparticles have also been used as delivery devices for viral antigens to stimulate an immune response[366] as well as co-stimulators for vaccines to help increase the immunological response[393] and establish immunological memory.[351]
- New tools to engineer and tailor drug delivery systems have been developed, examples include:
- applications of synthetic biology to construct biohybrid delivery materials;[349]
- design guidelines;[370]
- more sophisticated ways to build nanostructures;[371]
- the ability to build larger nanoparticles;[372]
- more refined controlled release;[372, 413, 549]
- enhanced drug loading;[372]
- the ability to build nano-metal oxide frameworks;[372]
- the ability to self assemble particles from increasing numbers of sub-units;[375]
- self assembling (and disassembling) systems;[376]
- collocation of drugs, ligands and other functionalities;[377]
- highly automated design strategies for complex nanostructures;[378]
- design guidelines to increase the penetration of targeted nanoparticles.[379]

5.3 Dispersal and delivery of biological agents

Advances in several key areas could simplify the delivery of a biological weapon:

- **Nanotechnology.** A wider range of nanoparticles, of different sizes, can more efficiently deliver complex payloads to diverse targets. Nanoparticles can now target previously inaccessible physiological sites and cell types (e.g. by crossing the blood-brain barrier). Nanoparticles also add other desirable characteristics to agents, such as increased persistence in the body and immune avoidance. Nanoparticles suitable for aerosol release have been developed since the Seventh Review Conference;
- **Aerobiology.** Offers powerful tools for modelling the release of bioweapons, including both environmental and indoor dispersal patterns, helping optimise the release of an agent. There have also been advances in equipment for generating and modelling aerosol dispersion have also been developed;
- **Use of chemical co-factors to increase the uptake of biological agents.** These have been identified for use with biologically active proteins; and
- **Increasing capacity to deliver biological weapons via the alimentary route.** The use of sophisticated formulations can improve absorption from the gastro-intestinal tract.

5.3.1 Aerobiology and modeling a release

- Tools for simulating how an agent reacts in the environment have improved, for example, an integrated model of environmental transport and human health exposure to biological pathogens in an indoor setting[466] or modelling the impact of architectural design on the movement of biological agents.[552]
- There have been developments in studying aerosol releases in the environment, for example, improved tagging mechanisms.[553]
- Drug delivery particles optimised for aerosol release have been developed[376, 381] as well as nebulisers and other devices for their delivery.[412]

5.3.2 Targeted and improved delivery

- Nanoparticles and drug delivery platforms have led to an increasing range of targeting options,[413, 554] examples include:
  - neurological targeting;[376]
  - targeting based upon genetic differences, such as SNPs;[400]
  - active and passive targeting;[401]
  - targeting to specific organs or cells;[385, 403, 551, 555]
  - cell-type-specific targeting for siRNA-carrying nanoparticles.[172]
- Improved viral vectors for gene therapy have been identified, including the synthesis of ancestral Adeno-associated virus vectors.[385]
- There have been developments that enable more precise targeting, examples include:
  - improved control over the placement of targeting ligands and nanoparticles and carriers;[377]
  - improved capabilities to design and build antibody-drug conjugates;[556]
  - engineered biomaterials for targeting;[351]
  - the development of polymeric nanoparticles for targeting;[399]
  - communication between nanoparticles to improve targeting.[404]
- Research has demonstrated that chemical cofactors can be used to increase the uptake of proteinaceous drugs.[557]

5.3.3 Alimentary delivery

A 2013 review highlighted 'increasing capacity to deliver biological weapons via the alimentary route'.[493]

6

Advances that increase risks relevant to the BWC

There have been a number of developments that increase overall risks relevant to the BWC by increasing capacity to circumvent detection, response, control and forensics capabilities.

These include:

- The use of the 'dark-web' and other information technology platforms to enable the proliferation of biological resources for prohibited uses, for example, the convictions for attempts to acquire, and the acquisition of toxins such as Ricin and Abrin.[558] A recent report noted that 'the rise in biotechnology e-commerce will significantly disrupt the effectiveness of current [export control] efforts'.[559]
- The potential to develop pathogens from parts not on any control list, or not traditionally considered to be pathogenic, for example using CRISPR/CAS9 mediated gene drives;[508, 509]
- The potential to alter the receptor binding specificity of key toxins, such as the anthrax toxin to provide 'receptor-redirected forms',[66] which could complicate identification and therapeutic intervention;
- Alterations to pathogens which 'will make it difficult to identify them by existing means of identification';[560]
- Alteration to pathogens 'which will lead to the failure of traditional prevention and treatment of infectious diseases and make efficient prevention and control more difficult';[213]
- It is now possible to sequence a virus, email its sequence, alter its physical properties and synthesise an altered agent at a second physical location faster than it is possible to ship and alter such an agent.[336, 337] This reduces the window for identifying and interdicting prohibited activities and alters the footprint of relevant activities and increases the importance of intangible technologies;
- The use of CRISPR/CAS9 genome editing to alter the drug susceptibility of a key vector for the transmission of an infectious disease[439] which offers an alternative mechanism for negating the efficacy of interventions to treat or control the spread of disease;
- The presence of virtual biotechnology companies[540] demonstrating the potential for complete outsourcing design, scale up and production of biological agents, increasing the importance of considering intangible technologies, altering the footprint of relevant activities and enabling increased compartmentalisation and geographic distribution of prohibited activities; and
- The potential for advances in 3-D printing to be used to:
  - make laboratory equipment, including controlled items 'offers a means to subvert traditional controls on process equipment made of high performance and corrosion resistant materials';[561]
  - print biological tissue, 'potentially offered actors the possibility of lower production costs and materials used and, significantly, a smaller footprint for production'.[561]

Appendices

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Topics covered during the intersessional process 2012 – 2014

2015

Producing and stockpiling biological agents

Dispersal and delivery of biological agents

2014

Pathogenicity and virulence

Transmissibility and host range

Toxins

Immunology and host-pathogen interactions

Antimicrobial and drug resistance

2013

Detection technology

Diagnosis and surveillance

Drugs (Including vaccines and therapeutics)

Responses

Annotated references

(Endnotes)

1 In May 2012, Iami *et al* reported having inserted a key binding structure from H5N1 influenza (that can infect but not spread in humans) into a H1N1 backbone. The authors identified four specific mutations to the binding structure that enabled the recombinant virus to spread via aerosols in ferrets.

2 In June 2012, Herfst *et al* reported having used site-directed mutagenesis and serial passage in ferrets to confer airborne transmission on the H5N1 influenza virus. The authors identified five specific mutations that confer airborne transmissible in ferrets.

3 In June 2012, Russell *et al* reported having used sequence data collected from disease surveillance efforts to map the current circulation of mutations known to affect mammal –to-mammal transmission in H5N1 influenza virus. The authors determined that two out of five known mutations are currently circulating and used modelling to 'to study factors that could increase and decrease the probability of the remaining substitutions evolving after the virus has infected a mammalian host'. The authors identified the specific mutations affecting transmission.

4 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'research in selection of strains with altered host specificity and/or high pathogenicity' including 'A number of scientists published their research findings on enteroviruses type 71 (Australia), influenza A H1N1 virus and superinfections agents (USA) and Dengue fever virus (Brazil)'.

5 In May 2013, Zhang *et al* reported having produced 127 reassortments of H5N1 and H1N1 viruses to create pathogens capable of mammalian aerosol transmission. The authors identified 'the H1N1 virus genes encoding acidic polymerase and nonstructural protein made the H5N1 virus transmissible by respiratory droplet between guinea pigs without killing them. Further experiments implicated other H1N1 genes in the enhancement of mammal-to-mammal transmission, including those that encode nucleoprotein, neuraminidase, and matrix, as well as mutations in H5 HA that improve affinity for humanlike airway receptors'.

6 In April 2014, Sutton *et al* reported having used serial passage of the virus in ferrets to produce a highly pathogenic H7N1 influenza virus capable of aerosol transmission in mammals. The authors identified four specific internal mutations and a single mutation in the stalk region of the hemagglutinin protein which together conferred airborne transmission. The authors determined that this 'transmission was not associated with loss of virulence'. They concluded that 'avian influenza virus can be adapted to become capable of airborne transmission in mammals without mutations altering receptor specificity. Changes in receptor specificity have been shown to play a role in the ability of avian influenza viruses to cross the species barrier, and these changes are assumed to be essential. The work reported here challenges this paradigm, at least for the influenza viruses of the H7 subtype'.

7 In October 2014, Zhou *et al* reported having used a replicative synthetic virus to characterize an uncultivable bat influenza virus. Whilst the authors were unable to rescue the wild type bat virus, they did rescue a modified bat-influenza virus that had the HA and NA coding regions replaced with those of H1N1. Their chimeric virus replicated efficiently in vitro and in mice, resulting in severe disease. By manipulating the HA and NA coding regions they identified a novel pathogenicity factor. Further studies enables the authors to both attenuate and increase the pathogenicity of their mutated virus. The authors demonstrated 'that bat-influenza has very limited genetic and protein compatibility with Type A or Type B influenza viruses'. The asserted that they 'pose little, if any, pandemic threat to humans'.

8 In June 2014, Wanatabe *et al* reported having created a novel influenza virus that resembles the causative agent of the 1918 pandemic by stitching together avian influenza segments. The authors then characterised the virus, demonstrating increased pathogenicity (when compared to any of the avian influenza viruses that donated parts) in mice and ferrets. The authors identified seven specific mutations needed to confer mammalian respiratory droplet transmission to the virus. This research was also highlighted in an August 2014 working paper submitted by the United States of America to the BWC Meeting of Experts, the authors of which noted that the recombinant virus was more virulent than any of the donor viruses and resembled the virulence of the 1918 pandemic strain.

9 In October 2014, Wei *et al* reported that 'an engineered reassortant swine influenza virus, with the same gene constellation pattern as the pandemic H1N1/2009 virus and subjected to only nine serial passages in pigs, acquired greatly enhanced virulence and transmissibility'. The authors identified a series of mutations 'able to confer respiratory droplet transmission as effectively as the pandemic H1N1/2009 virus'. They concluded 'pigs can readily induce adaptive mutational changes to a precursor pandemic-like virus to transform it into a highly virulent and infectious form akin to that of the pandemic H1N1/2009 virus'.

10 In September 2013, Shi *et al* reported the structures and receptor binding of hemagglutinins from human-infecting H7N9 influenza viruses. The authors identified a single mutation that allowed the virus to bind to both avian and human receptors. The results published convinced the authors that 'that other amino acid substitutions contribute to the receptor-binding switch'.

11 In October 2013, Knepper *et al* reported having identified a H7N9 influenza virus isolate adapted for efficient growth in human lung tissue. The authors evaluated the replication, tropism, and cytokine induction of the virus, demonstrating that it 'replicated similarly well as a seasonal IAV in explanted human lung tissue, whereas avian H7 subtype viruses propagated poorly'.

12 In December 2013, Hai *et al* reported the H7N9 influenza virus gains neuraminidase inhibitor resistance without loss of *in vivo* virulence or transmissibility. The authors also identify the specific mutations involved.

13 In November 2014, Dowall *et al* reported having used serial passage of Ebola virus in guinea pigs to identify a number of mutations associated with host range. The authors also identified 'genome modification and coding changes that are associated with increasing virulence, pathogenesis and disease pathology'.

14 In May 2013, Zhang *et al* reported the atomic structures of H5 influenza virus Hemagglutinin (HAs). The authors 'determined the complex structures of wild-type and mutant HAs derived from an Indonesia H5N1 virus bound to either avian or human receptor'. They demonstrated the 'structural and biophysical basis for the H5N1 adaptation to acquire human, but maintain avian, receptor-binding properties'.

15 In June 2013, Zhang *et al* reported the conformational changes necessary for the H5N1 influenza virus to alter its receptor-binding preference from avian to human receptors. The authors provided the 'structural and biophysical basis for the H5N1 adaptation to acquire human, but maintain avian, receptor-binding properties'. In December 2013, Xu *et al* reported that whilst these receptors were capable of binding with human receptors, that they bound preferentially with avian receptors. The authors concluded 'the current human H7N9 viruses are poorly adapted for efficient human-to-human transmission'.

16 A July 2012 working paper by China for the BWC Meeting of Experts highlighted 'confirmation of the correlation between genetic variation and disease sensitivity makes it possible to improve the specific microbes' pathogenicity, infectivity, and host specificity using combinatorial approaches of synthetic biology, reverse genetics and whole genome in vitro directed evolution'. The authors also noted 'revelation of pathogenic microbes' genome evolution and its relation with infectivity and pathogenicity and greatly enhance the surveillance, diagnosis and therapy of related infectious diseases. There is no doubt that such DNA sequence information can also be used for the modification of antigenicity, infectivity, toxicity and drug resistance of traditional pathogens, even for the artificial design and synthesis of totally new pathogens, which will lead to the failure of traditional prevention and treatment of infectious diseases and make efficient prevention and control more difficult'.

17 In November 2012, Streicker *et al* reported the evolutionary routes rabies virus took to establish itself in a new host following repeated host shifts among bats. The authors demonstrated 'that although rabies viruses shared consistent three-stage processes of emergence in each new bat species, host shifts involving greater numbers of positively selected substitutions had longer delays between cross-species transmission and enzootic viral establishment'. The authors noted that there 'are multiple evolutionary routes to host establishment in a zoonotic RNA virus that may influence the speed of viral emergence'.

18 A 2014 review of the application of tissue engineering approaches to the modelling of viral infections highlighted progress in overcoming shortcoming in other model systems, noting "Standard tissue culture models lack critical emergent properties driven by cellular organization and *in vivo*–like function, whereas animal models suffer from limited susceptibility to relevant human viruses and make it difficult to perform detailed molecular manipulation and analysis". The authors conclude recent developments in tissue engineering "faithfully recapitulate the *in vivo* viral life cycle".

19 In August 2011, Rasko *et al* reported having sequenced the organism for a particularly lethal *Escherichia coli* (O104:H4). The authors used the sequence data to provide insights into the origins of the bacteria and core aspects of its pathogenicity.

20 A January 2012 review of biomedical applications of synthetic biology highlighted development for understanding pathogen mechanism, including for influenza, SARs and HIV.

21 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'Findings of the study on orthoreoviruses and influenza A (People's Republic of China), brucellosis (Spain) were published' as well as 'Italian scientists… studying the formation of Shigella (dysentery agent) pathogenic strains by means of loss of some genetic elements of *Escherichia coli*. Such a mechanism can significantly increase the hazardous properties of entero-haemorrhagic *Escherichia coli* (O104: H4)'. The authors also took note of 'research in selection of strains with altered host specificity and/or high pathogenicity' including 'A number of scientists published their research findings on enteroviruses type 71 (Australia), influenza A H1N1 virus and superinfections agents (USA) and Dengue fever virus (Brazil)'.

22 An August 2014 working paper submitted by the United States of America to the BWC Meeting of Experts highlighted research:

- Enabling the prediction of the virulence of an emerging pathogen from its sequence information. The authors highlighted recent work on methicillin-resistant *Staphylococcus aureus*. The authors noted 'such knowledge would improve and hasten development of vaccines, therapeutics, and diagnostics with increased specificity for virulence factors. Such knowledge could also enable mitigation of the host immune response, turning it up or down to minimize host damage';
- Identifying how varicella zoster virus (VZV) manipulates the host immune system. That authors noted there are 'several mechanisms by which VZV proteins prolong infection, including: (i) promoting survival of infected T cells and skin cells, (ii) enabling VZV to go unrecognized by the immune system, and (iii) inducing "gene silencing" that enables the virus to go dormant in neurons';
- On virulence factors contributing to persistent infections. The authors noted recent research with *Mycobacterium tuberculosis* (Mtb) 'to identify potential virulence factors in the pathogen's genome in order to understand the ability of Mtb to survive for long periods in human lungs. This research suggested Mtb enzymes as virulence factors that allow survival within host cells under different *in vivo* conditions'.

23 In October 2014, Ayllon *et al* reported a single specific mutation that increased the virulence of H7N9 influenza virus.

24 In January 2015, Bi *et al* reported having identified specific mutations to a series of internal genes of H7N9 influenza virus that increase the pathogenicity of the virus in vitro and *in vivo*.

25 In August 2013, an international group of authors published their intent to study the host range, virulence and transmission of H7N9 influenza viruses as well as to evaluate the effectiveness of antiviral drugs and vaccine candidates. The authors provided the rationale for conducting such work.

26 In December 2014, Jeon *et al* reported that B. anthracis DNA may contribute to anthrax pathogenesis by enhancing LT activity via cytokines mediated by toll-like receptors.

27 In February 2015, Hubbard *et al* reported have used transcriptomics approaches to gain insights into wheat yellow rust populations in the UK. The authors demonstrated a major shift in the causative agent in recent years – with four distinct lineages being tracked and correlating to different phenotypes identified by previous virulence assays. The authors noted that the methods described 'circumvents the difficulties associated with obligate plant pathogens. In principle, this strategy can be widely applied to a variety of plant pathogens'.

28 In September 2014, Morrison *et al* reported having characterised the host immune response to H7N7 and compared it to those of H5N1, H1N1 and the 1918 influenza viruses and identified a common transcription signature connected to pathogenicity. The authors screened this signature against existing drugs and identified six FDA-approved drugs that could potentially be repurposed as H7N9 influenza therapeutics.

29 In February 2011, Dubey and Ben-Yehuda reported having identified a novel signalling mechanism in bacterial populations. The authors identified intercellular nanotubes and demonstrated that bacteria use them to exchange cytoplasmic molecules, non-hereditary antibiotic resistance and plasmids. The demonstrated that 'nanotubes also formed in an interspecies manner, between B. *subtilis* and *Staphylococcus aureus*, and even between B. *subtilis* and the evolutionary distant bacterium *Escherichia coli*'.

30 In February 2012, Soanes *et al* reported a high-resolution analysis of gene expression changes associated with rice blast infections in plants. The authors mapped and analysed global patterns of gene expression during appressorium (a specialised infection structure) development. They demonstrated gene expression changes over time and revealed the activity of key regulators.

31 In June 2012, Loquet *et al* reported the structure of the type III secretion system used by pathogens to manipulate host cells. The authors reported compiling the complete atomic structure of the *Salmonella typhimurium* T3SS needle.

32 In May 2013, Zhao *et al* reported the structure and molecular dynamics of a mature HIV-1 capsid. The authors used cryo-electron microscopy and cryo-electron tomography to identify the structure of the protein assembly and the its core, respectively. They used the data to produce an all-atom model of the capsid. Mutagenesis studies demonstrated that the hydrophobic structures in the core were 'crucial for capsid assembly and stability, and for viral infectivity'.

33 In July 2013 Zhou *et al* reported the biological features of a novel strain of H7N9 influenza proving fatal to humans. The authors demonstrated that the virus could bind to both human and avian receptors. The also detailed its ability to infect human tissues, partially characterised the host response and provided insights into its pathogenicity.

34 In July 2013, Coggeshall *et al* reported a new model to explain the pathology of inhalational anthrax. The authors use their model to 'propose that death in inhalation anthrax results from an overwhelming bacteraemia that leads to severe sepsis', rather than from toxaemia.

35 A March 2014 review outlines progress in understanding life cycle of *Yersinia pestis*, focusing in particular on how the bacteria 'survive and suppress the host immune response during the initial phase of the infection when the bacteria have not been acclimated to the mammalian host temperature' necessary for the expression of virulence factors.

36 A July 2011 review of microbial genomics and infectious diseases highlighted that 'the revolution in DNA-sequencing technology has to a large extent democratized microbial genomics and altered the way infectious diseases are studied... Today, the major challenges in microbial genomics are to predict the function of gene products and the behaviour of organisms and communities from their sequences and to use genomic data to develop improved tools for managing infectious diseases'. The author highlighted progress in cataloguing genomic diversity, molecular epidemiology, understanding pathogenesis, metagenomics, pathogen discovery and diagnostics, therapeutics and drug discovery, and vaccines.

37 In May 2012, OpGen announced the formation of 'a public health consortium to evaluate... Whole Genome Mapping technology for strain typing microorganisms that cause health outbreaks'. They noted that 'comprehensive structural analysis of microbial genomes... combined with sequencing, [creates] a new genetic analysis workflow... that more accurately detects genetic elements associated with virulence and drug resistance'.

38 In January 2012, Strecher *et al* reported that gut inflammation can boost horizontal gene transfer between pathogenic and commensal *Enterobacteriaceae*. The authors demonstrate that 'pathogen-driven inflammatory responses in the gut can generate transient enterobacterial blooms in which conjugative transfer occurs at unprecedented rates. These blooms may favour reassortment of plasmid-encoded genes between pathogens and commensals fostering the spread of fitness-, virulence-, and antibiotic-resistance determinants'.

39 In February 2012, Soares *et al* reported having developed a software suite to identify pathogenicity islands that 'harbour clusters of virulence genes that mediate the adhesion, colonization, invasion, immune system evasion, and toxigenic properties'. The authors demonstrated their software package was more accurate than existing tools. The used the software to identify seven putative pathogenicity islands in the animal pathogen *Corynebacterium pseudotuberculosis*.

40 A January 2014 review of computational approaches to identifying pathogenicity islands highlighted that they 'have some detectable properties, such as having different genomic signatures than the rest of the host genomes'. The authors noted progress in computational approaches to identifying them.

41 A July 2012 presentation by Japan to the BWC Meeting of Experts highlighted the dual use potential of recent developments, including in:

- Synthetic biology enabling 'virus development/modification using artificial/modified genetic information' as well as more efficient integration of disease causing and toxin related genes;
- Bioinformatics, increasing the efficiency of comparing and retrieving data related to pathogencity and toxins;
- Systems biology, assisting in 'estimating effects of disease-causing agents on human cells' functions' and more efficient analysis of disease causing agents and opportunities to increase the damage done to the target.

42 An August 2013 review of the genomics of emerging pathogens highlighted "advances in methods for microbial discovery and characterization, as well as strategies for testing the clinical and public health significance of microbe-disease associations".

43 In January 2015, Yoon *et al* reported the creation of a database to house details of pathogenicity islands and resistance islands. The authors report '223 types of PAIs with 1331 accessions, and 88 types of REIs with 108 accessions'.

44 In January 2013, Varela *et al* reported having used a synthetic biology approach for the rapid rescue and manipulation of Schmallenberg Virus and developed a mouse model to study infection. The authors demonstrated that one of the non-structural proteins protein encoded in the virus blocks a key part of the host's innate immune system. They also demonstrated that serial passages in cell culture increased the virulence of their cloned virus in mice.

45 In October 2013, Pechous *et al* reported the early host-pathogen interactions of *Yersinia pestis* that leads to the progression of pulmonary infection in pneumonic plague. The authors describe a two-step progress with 'an initial pre-inflammatory phase facilitating bacterial growth in the absence of host inflammation, followed by a pro-inflammatory phase marked by extensive neutrophil influx, an inflammatory cytokine storm, and severe tissue destruction'.

46 In March 2015, Sivaraman *et al* reported a novel cytokine activation which occurs early after bacteria enter the lung, and which 'eventually contributes to pulmonary inflammation and pathology during the later stages of infection'. The authors also demonstrate 'Y. pestis also activates the induction of IL-1 receptor antagonist... and this activation likely contributes to the ability of Y. pestis to establish the initial pre-inflammatory phase of disease'.

47 In April 2014, Caulfield *et al* reported a novel mechanism through which *Yersinia pestis* manipulates innate immunity during pneumonic plague. The authors demonstrate that Y. pestis degrades an apoptotic signalling molecule, thereby altering the host inflammatory responses, enables enhanced Y. pestis growth in the lungs, and enabling its full virulence.

48 An October 2014 review of the convergence of biology and chemistry highlighted:

- 'The technical capability for chemical synthesis of toxins exists, and synthetic biology approaches could also be used for toxin production and modification'. The authors noted recent research with Saxitoxins;
- 'Growing research interest in toxins for applications in medical treatment, life sciences research, pharmaceuticals, and agriculture shows that technological advances are changing the way in which toxins are being produced and used'.

49 In August 2011, Moreau *et al* reported genome-wide RNAi screens identifying genes required for intoxication from Ricin and Pseudomonas exotoxin. The authors demonstrated striking differences in the pathways used by the two protein toxins.

50 In May 2011, Carette *et al* reported 'global gene disruption in human cells to assign genes to phenotypes by deep sequencing'. The authors demonstrated that this approach could be used to 'identify 743 mutations distributed over 12 human genes important for intoxication by four different CDTs' (cytolethal distending toxins).

51 In February 2012, Martchenko *et al* reported that human genetic variation alters anthrax toxin sensitivity. The authors demonstrated that extensive human diversity impacts toxin binding and uptake, which in turn alters cell lethality. They identified individual differences in a key binding domain expression levels which was a 'determinant of this diversity'.

52 In December 2013, Vonk *et al* reported having 'sequenced the genome of a venomous snake, the king cobra, and assessed the composition of venom gland expressed genes, small RNAs, and secreted venom proteins'. The authors demonstrated 'dynamic gene evolution and adaptation in the snake venom system'.

53 A June 2014 review of Botulinum neurotoxins (BoNT) highlighted 'recent studies that have improved our understanding of the genetics and structure of BoNT complexes... [and] recent insights into the mechanisms of BoNT entry into the general circulation, neuronal binding, membrane translocation and neuroparalysis.

54 In February 2013, Bassik *et al* reported a two-stage strategy for developing genetic interaction maps and then applied it to Ricin. Genetic interaction maps measure of how strongly the function of one gene depends on the presence of a second. They enable 'the systematic exploration of gene function in microorganisms'. The authors demonstrate that the Ricin map 'broadly recapitulates known pathways' but 'provides many unexpected insights' including 'a noncanonical role for COPI, a previously uncharacterized protein complex affecting toxin clearance, a specialized role for the ribosomal protein RPS25, and functionally distinct mammalian TRAPP complexes'.

55 In September 2011, Pincus *et al* reviewed the cellular mode of action, clinical symptoms and pre- and post-exposure approaches to prevent intoxication from Ricin toxin.

56 In September 2011, Herper *et al* reported using fluorescence and electron microscopy to reveal how Botulinum neurotoxins are taken up at the pre-synaptic nerve terminal. The authors used these insights to identity a compound that blocks the mechanism. They found that this compound, a novel potent Dynasore analog, conferred a 'significant delay of >30% in the onset of botulism... in mice'. In October 2013, McCluskey *et al* reported having improved the efficacy of Dynadore analogues.

57 In January 2013, Orr *et al* reported having characterized the gene involved in the second step of Saxitoxin biosynthesis.

58 In May 2013, Gregers *et al* reported that overexpression of the endoplasmic reticulum chaperone BiP 'inhibited Ricin translocation and protected cells against the toxin. Furthermore, shRNA-mediated depletion of BiP enhanced toxin translocation resulting in increased cytotoxicity'.

59 In July 2013, Bagaria *et al* reported insights into the mechanism of action of the only monoclonal antibody demonstrated to neutralize the Abrin toxin.

60 In September 2013, O'Hara and Mantis reported the mechanism of action of antibodies against both Ricin chains.

61 In October 2013, Dover *et al* reported the molecular characterization of the novel botulinum neurotoxin. The authors demonstrated that the gene sequence was significantly different from previously identified toxin types but did not publish the full sequence.

62 In April 2014, Dong *et al* reported characterising the absorption, distribution and pathological injury in mice due to ricin poisoning via the alimentary pathway.

63 A May 2015 review of the use of botulinum toxin as a painkiller highlighted recent research that revealed that the detailed structural bases of these neurotoxins interactions with their cellular receptors. The authors noted progress in understanding the molecular and cellular mechanisms related to the efficacy of botulinum toxins.

64 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted 'the production of toxic chemicals, including toxins, through biological synthesis (an aspect of the convergence of chemistry and biology); encapsulation and delivery through nanotechnology; and flow microreactors, which enable types of chemical reactions under conditions that were not previously technically feasible'.

65 An August 2014 presentation by the OPCW to the BWC Meeting of Experts highlighted enabling technologies having resulted in 'an expanded capability to redesign or manipulate organisms' and 'to design and engineer improved enzymes'. The authors noted metabolic pathways 'have been published for production of Saxitoxin, ricin… and many other toxins'. They highlighted that 'in vitro biosynthesis of Saxitoxin and ricin has been described' but that 'there are practical limitations with regard to scale and complexity. Obtaining ricin and small quantities of Saxitoxin from their natural sources is simpler than employing metabolic engineering strategies'.

66 In May 2012, Mechaly *et al* reported having changed the Receptor specificity of anthrax toxin. The authors the receptor specificity of the transport protein of anthrax toxin may be readily changed, raising the possibility that receptor-redirected forms of protective antigen (PA) and PA homologs may be useful for research and medical applications requiring modification or ablation of designated populations of cells'.

67 In October 2013, Barash and Arnon reported discovering a new type of toxin from *Clostridium botulinum* – the first to be discovered in more than 40 years. The authors used a mouse bioassay to demonstrate the new isolated toxin was different from known types. This research was highlighted in an August 2014 working paper submitted by the United States of America to the BWC Meeting of Experts, where the authors noted 'immunological approaches indicated that antibodies to known botulinum toxin (types A through G) did not react with the new toxin; genome sequencing revealed its similarities and differences to other botulinum toxins; and animal studies demonstrated its extreme toxicity as well as the potential for vaccine development'.

68 In December 2013, Redmann *et al* reported having developed 'a robust high-content screen was developed to discover novel compounds that stabilize intracellular Ricin and limit Ricin intoxication'. The authors focused on compounds that target Ricin toxin chain-A retrograde translocation.

69 In October 2013, Ferrari *et al* reported having combined the botulinum type A protease with the tetanus binding domain, which natively targets central neurons. The parts, although produced separately, were joined using site-specific protein stapling. The authors demonstrated that the synthetic molecule was able to block pain in a rat model and neuron activity in the visual cortex.

70 In September 2012, Hu *et al* reported having developed and characterized a monoclonal antibody capable of neutralizing the Ricin toxin. The authors demonstrated that it could rescue 100% of mice injected with a low dose of the toxin after 4 hours post exposure and 50% of mice 6 hours post exposure.

71 In September 2012, Yermakova *et al* reported having identified sub-domains of Ricin's B subunit as targets of toxin neutralizing and non-neutralizing monoclonal antibodies.

72 In December 2012, Saito *et al* reported having synthesized and tested a number of competitive active-site inhibitors of Ricin Toxin A. The authors studied the binding dynamics involved and identified a number of additional key factors not predicted by modelling.

73 In April 2013, Song *et al* reported that antibodies against Ricin A Chain offered greater protection than those targeting the B Chain. Ricin A Chain antibodies hinder intracellular routing of toxin and protect cells even after toxin has been internalized.

74 In September 2013, Yermakova and Mantis reported that monoclonal antibody fragments 'neutralize Ricin in a cell based assay, and in a mouse challenge model as effectively as their respective full length parental IgGs'.

75 In December 2013, Vance *et al* reported having identified monomeric single-chain camelid antibodies capable of neutralizing Ricin *in vitro* and engineered a heterodimeric version that neutralized Ricin *in vivo*.

76 In January 2014, Hu *et al* reported using intact IgG from goat anti-ricin hyperimmune sera to provide immediate protection following ricin exposure and to confer an active immunity against ricin that subsequently results in long term protection (up to 5 months).

77 In April 2014, Yermakova *et al* provided additional functional information about antibody mediated neutralisation of ricin.

78 An August 2014 presentation by Germany to the BWC Meeting of Experts highlighted advances in assessing the quality of detection technologies for toxins. The authors noted existing shortcomings at the national and laboratory level and identified technical deficits including needs for: detection and identification criteria for individual methods; accepted SOPs/ROPs for different technological approaches; closing analytical detection gaps; reference material; reagents and methods; exchanges of know-how; training capabilities; and regular ring trials with increasing level of difficulty on selected techniques.

79 In 2011, the US National Academies of Sciences published a report reviewing the threat posed by fungal diseases to human, animal and plant health. The report was derived from a public meeting on this issue, the first ever held by their Forum on Microbial Threats. The report addresses fungi as pathogens, noting their ability to 'cause disease in healthy humans and animals' as well as to 'endure adverse environmental conditions and thrive outside their host'. The impact on plants was also stressed, noting that fungal plant pathogens had been responsible for the Irish Potato Famine, the Southern Corn Leaf Blight epidemic of the 1970s as well as Dutch Elm disease.

80 In November 2013, Li *et al* reported having identified three genes associated with infections of *Plasmodium falciparum* (the causative organism of malaria) in the vector mosquito, Anopheles gambiae.

81 In August 2012, Grizenkova *et al* reported that the overexpression of a gene associated with heat shock proteins could reduce the incubation of prion diseases. The authors demonstrated that an eight fold overexpression of the gene could reduce the incubation time by up to 16%.

82 In February 2013, Ciaiti *et al* reported that the mis-folded proteins responsible for neurodegenerative prion diseases play an important role in brain development, in particular regulating synaptic plasticity in the developing hypocampus.

83 In March 2013, Karapetyan *et al* reported having developed and used a new screening process to identify two therapies against prion diseases. The authors demonstrated that two drugs already approved for human use (astemizole and tacrolimus) reduce cell-surface prion proteins and inhibited prion replication in neuroblastoma cells. Tacrolimus reduced total cellular prion protein levels by a nontranscriptional mechanism. Astemizole stimulated autophagy, a hitherto unreported mode of action for this pharmacophore. Astemizole, but not tacrolimus, prolonged the survival time of prion-infected mice'.

84 In March 2012, Marazzi *et al* reported a novel mechanism by which influenza viruses suppress the host's anti-viral response. The authors described a mechanism by which the influenza virus uses a non-structural protein to interfere with the host cell's epigenome. The viral protein mimics a key regulator reducing antiviral gene expression and rendering cells more susceptible to viruses.

85 In April 2012, Everitt *et al* reported that a set of proteins known to inhibit the replication of certain pathogenic viruses play an important role in protecting against severe viral pneumonia present in certain cases of influenza. They demonstrated that mutations in the genes responsible for these proteins reduce their antiviral activity and can profoundly alter the course of influenza infections in humans and mice.

86 In August 2014, Yoshizumi *et al* reported the mechanism by which Influenza A virus protein PB1-F2 impairs innate immunity.

87 An August 2014 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlights:

- Pathogen-specific mechanisms for avoiding the immune system, including for vaccinia virus;
- Dengue virus' ability to hide from host immune defences by avoiding recognition by components of the innate immune system through the use of enzyme known as MTase to 'chemically modify its genetic material and escape detection';
- Severe acute respiratory syndrome coronavirus (SARS-CoV)'s use of the enzyme PLpro (or papain-like protease) to remove host cell proteins involved in triggering the innate immune response;
- 'An outer protective layer formed of capsular polysaccharides' used by *Escherichia coli* and other pathogens 'which prevents them being recognised and destroyed by the host immune system';
- Antigenic variation, used by viral, bacterial, fungal and parasitic pathogens to sidestep antibodies created in response to previous infections;
- Modulation of the protein-protein interactions involved in the signalling pathway that activates an innate immune response. The authors highlight recent research suggesting that *Salmonella enterica*, *Brucella* sp., *E.coli* and *Yersinia pestis* all use this approach;
- The 'mechanism by which viruses such as influenza, West Nile and dengue activate a class of molecules, known as TAM receptors, which are central inhibitors of the innate immune response to pathogens';
- The mechanism through which '*Yersinia pestis*... disrupts the host immune system by interacting with a receptor protein on immune cells';
- The Type III secretion process used by many bacteria to hijack host cells by the injection of microbial proteins which 'assist their survival and block the host cell immune response';
- The recently discovered broadly-reactive antibody-binding protein used by *Mycoplasma* species 'to bind with high affinity to all types of human and non-human antibodies through attachment to conserved regions, thus blocking antibody-antigen binding';
- The mechanism used by pathogens such as *Y.pestis* to survive inside macrophages by degrading the compound itaconate, which interferes with bacterial metabolism.

88 In April 2012, Nham *et al* used bioluminescence imaging to track the course of *Yersinia pestis* infections in a living host. They demonstrated that the high variability of the kinetics of infection was determined by the time taken for the bacteria to move from the site of infection to the draining lymph nodes. They noted that when the infection reached this point, 'the disease progresses extremely rapidly, leading to the invasion of the entire body within two days and to death of the animals. This highlights the extraordinary capacity of Y. pestis to annihilate the host immune response'.

89 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'research in immunity overcoming strains' including 'studies of American and Italian scientists on the plague agent, including on the analysis and design of molecular mechanisms that enable overcoming both artificial (vaccine-generated) and natural human immunity'.

90 A September 2013 review highlighted progress in understanding the relationship between *Mycobacterium tuberculosis* and its host. The authors note 'The host resists infection by releasing damaging free radicals, pushing the pathogen towards lysosomal degradation, releasing an arsenal of cytokines for triggering adaptive immunity and facilitating apoptosis for effective T cell antigen presentation. The bacterium counters these mechanisms and also metabolically reprograms the macrophage to its own benefit'.

91 In February 2014, Akey *et al* reported the structures used by *flaviviruses* to avoid the host immune system.

92 In May 2012, Habets and Brockhurst reported having experimentally evolved *Staphylococcus aureus* to be resistant to 'human-neutrophil-defensin-1, a key component of the innate immune response to infection. This unintended consequence of therapeutic use could drastically undermine our innate immune system's ability to control and clear microbial infections'.

93 In September 2011, Teijaro *et al* reported a mechanism responsible for inducing cytokine storms in response to certain infections. The paper describes the cellular and signalling mechanisms responsible and demonstrates that it is possible to modulate them, reducing the innate immune response and offering potential therapeutic interventions to prevent cytokine storms. Elucidation of this mechanism may also offer opportunities to confer the ability to initiate cytokine storms to other pathogens.

94 In September 2013, Bradley-Stewart *et al* reported having identified cytokine concentrations associated with clinical outcome in patients infected with pandemic H1N1 influenza. The authors demonstrated 'a number of cytokines were found to be substantially elevated in patients with severe influenza' supporting earlier work 'suggesting a role for proinflammatory cytokines in influenza-induced lung pathology'. The authors also demonstrated that another cytokine 'was significantly lower in patients with severe infection suggesting it is actively suppressed. As EGF has a role in role in cell proliferation and tissue repair, it may protect the lung from host or virus mediated damage'.

95 A January 2012 review of biomedical applications of synthetic biology highlighted development for understanding immune systems, including B cells and autoantigens.

96 In February 2012, Martchenko *et al* reported that human genetic variation alters anthrax toxin sensitivity. The authors demonstrated that extensive human diversity impacts toxin binding and uptake, which in turn alters cell lethality. They identified individual differences in a key binding domain expression levels which was a 'determinant of this diversity'.

97 In March 2012, Moseman *et al* reported a previously unreported mechanism through which the innate immune system combats viral infections. In a second paper during the same month, Jaeger *et al* reported that neutrophils (the most common type of white blood cell) played a critical role in guiding antimicrobial natural killer cells to their targets. Disrupting the function of neutrophils was demonstrated to impair a host's ability to mount an effective response.

98 In June 2012, Lee and Groisman reported that a Salmonella virulence locus is controlled by an ATP-sensing leader messenger RNA. The authors noted the results 'indicate that pathogens can interpret extracellular cues by the impact they have on cellular metabolites'.

99 An October 2013 review of structural insights into plant–pathogen interactions highlighted advances in understanding the molecular mechanisms of plant disease. The authors noted 'genomic, bioinformatic, proteomic, biochemical and cell biological analyses of plant–pathogen interactions, three-dimensional structural studies of virulence proteins deployed by pathogens to promote infection, in some cases complexed with their plant cell targets, have uncovered key insights into the functions of these molecules'. The highlighted that 'structural information on plant immune receptors, which regulate the response to pathogen attack, is also starting to emerge'.

100 A January 2014 review of the nanoencapsulation of cells highlighted 'recent research and development of the systems of nano-thin layers coated cells for biomedical applications. Polyelectrolyte based nano-thin polymer coatings… are a promising part of the systems involving cells for biological processes regulation. The purpose of the layer-by-layer coating technique application is to minimize capsule void volume and separate cells from the host immunological system eliminating immunosuppressive therapy during transplantation'.

101 In February 2015, Pechous *et al* reported a novel bacterial mediator of pulmonary inflammation in pneumonic plague. The authors used *in vivo* transcriptional profiling to identify 'five Y. pestis genes that contribute to the development of pneumonic plague. Deletion of one of these genes, ybtX, did not alter bacterial survival but attenuated host inflammatory responses during late-stage disease'.

102 In May 2012, Feng *et al* reported biochemical basis of how one pathogen-injected effector protein inhibit plant immunity. The authors demonstrate the effector modifies two 'receptor-like cytoplasmic kinases known to mediate immune signaling', thereby reducing their kinase activity and consequently inhibiting downstream signaling. This effectively enhances virulence of this plant pathogen and inhibits plant immunity.

103 In January 2014, Hyde *et al* reported one evasion mechanism by which viruses use RNA structural motifs to avoid immune restriction.

104 In June 2012, Illing *et al* reported having used a drug used to treat HIV to modify the set of antigens that activates the immune system, triggering life-threatening auto-immune responses. This provides a critical insight into mechanisms responsible for hypersensitivities to small molecule drugs and a potential new agent as small molecule drugs could be used to initiate dangerous hypersensitivity reactions.

105 An August 2013 statement by Iran on behalf of the Group of NAM and other States Parties to the BWC highlighted that 'advances in enabling technologies like bioinformatics; computational biology; DNA microarrays; gene synthesis technology; high-throughput mass spectrometry; high-throughput sequencing; nanotechnology; synthetic biology; systems biology; and whole-genome directed evolution are critical for future life sciences research and development. These enabling technologies have many benefits in faster, cheaper, and easier application of biological science and technology for both public health and security purposes, increased capacity and better understanding of disease and healthcare technologies by more people in more locations throughout the world'. The same statement was repeated at the August 2014 BWC Meeting of Experts and the December 2014 BWC Meeting of States Parties.

106 In October 2014, Andoh *et al* reported having created a fully functional atomic scale molecular model of the capsid of Foot-and-Mouth Disease virus. The used the model to successfully explain physical characteristics of the virus.

107 In January 2015, Han *et al* reported modelling the three-dimensional structure of foot-and-mouth disease virus and its biological functions. The authors reported significant insights into 'virus assembly and dissociation, formation of capsid-like particles and virus-receptor complexes, and viral penetration and uncoating'.

108 A July 2012 presentation by Mexico to the BWC Meeting of Experts highlighted the use of metagenomics for the identification of organisms present in a mixed environmental sample. They noted its use in identifying and tracking a specific agent. The author provided an example of the use of metagenomics approaches to study the cerebrospinal fluid of patients with meningoencephalitis.

109 In July 2012, Dreyfuss *et al* reported having mathematically derived the absolute limits of heritability and prevalence of predicting disease course using genetic factors. They argue that determining 'such limits is valuable in understanding the implications of genetic testing even before additional associations are identified'.

110 In September 2011, Suhre *et al* reported 'a comprehensive analysis of genotype-dependent metabolic phenotypes using a GWAS with non-targeted metabolomics'. The authors 'identified 37 genetic loci associated with blood metabolite concentrations' and noted these 'associations provide new functional insights for many disease-related associations that have been reported in previous studies'.

111 A July 2012 presentation by Pitt to the BWC Meeting of Experts highlighted a range of advances and technologies in the life sciences, including distributed computing technologies to address bioinformatics and big data challenges.

112 A March 2012 review of structural analysis of receptors highlighted a series of technological developments to address the challenges of being able to use x-ray crystolography to determine the structures of G-protein-coupled receptors ('ubiquitous cell-surface molecules that are activated by light, odours, hormones and neurotransmitters'). The authors describe how proteins are used to stabilize restive sections of the receptor and a special 'medium called the lipidic cubic phase' to enable the receptors to pack into crystals. In May 2012, Wu *et al* reported using these developments to determine the structure of opinoid receptors in humans.

113 In April 2014, Owen *et al* reported significant progress in room-temperature macromolecular crystallography. This is an important tool in determining the physical structure of macromolecules.

114 An October 2013 review of optogenetics explore the history of how a bacterial sensing mechanism used to detect light had been used to turn off and on neurons in model organisms, such as mice. The ability to manipulate specific neurons provided a higher fidelity research tool to determine the function and role of those neurons and has proven valuable in studying what causes neuropsychiatric disorders and how brain diseases can be treated. Similar tools have also been used to determine the neurological characteristics of addiction.

115 In June 2014, Gunaydin *et al* reported a new methodology (fiber photometry) to "optically record natural neural activity… to elucidate the realtime role of specified pathways in mammalian behavior". The authors also demonstrated that manipulating neural patterns could induce certain types of behaviour.

116 A January 2014 review described progress in understanding "the concrete mechanistic distinctions between adaptive, physiological behavioural states and psychiatric-disease-related behavioural states". The authors review a series of "investigations have revealed that control of projection-specific dynamics is well suited to modulating behavioural patterns that are relevant to a broad range of psychiatric diseases".

117 A December 2014 review of developments in microscopy highlighted series of techniques to "render opaque tissue transparent. Their use helps to image, label and identify structures at single-cell resolution". The authors highlighted their use in imaging of structurally intact brain circuits

118 In December 2013, Shiromani *et al* reported having manipulated the activity of neurons to modulate symptoms associated with narcolepsy. The authors reported having stimulated neurons to induce sleep on demand.

119 A December 2012 review of the applications, challenges, and opportunities of optogenetics for psychiatry highlighted an increasing synergy between capabilities for modulation of defined neural projections with an ability to alter function along pathways of neural communication, an important characteristic of psychiatric disease. The authors review work demonstrating an ability to manipulate neurons to influence mood and behaviour (such as inducing violence and aggression) as well as mental health (such as inducing anxiety disorders such as PTSD) in mice.

120 An August 2014 commentary on reports of the use of CRISPR/CAS9 gene drives highlighted potential beneficial applications including 'reprogramming mosquito genomes to eliminate malaria, reversing the development of pesticide and herbicide resistance, and locally eradicating invasive species'.

121 A November 2013 review of emerging nanotechnology-based tools for biology and medicine, highlighted the potential to use nanopores 'patterned at high densities around single cells' to enable 'highly localized measurements at submicron and subcellular length scales'. The authors highlight potential applications to measure the cellular secretion of growth factors, cytokines, and other signalling molecules into extracellular spaces.

122 In October 2013, Yeow *et al* reported on the 'fabrication, characterization, and testing of a polymer microprojection array, for the direct and selective capture of circulating biomarkers from the skin of live mice'. The authors demonstrated in vitro and *in vivo* results comparable with other more expensive and complex arrays.

123 A July 2012 working paper by China for the BWC Meeting of Experts highlighted 'Mass spectrometry... can also be used for high-throughput nucleic acids analysis, which is particularly useful for the detection of uncultivable microbes. Hence, the development of high-throughput MS technologies will facilitate the surveillance and diagnosis of BWC relevant agents, and forensic medicine'.

124 In January 2014, Organovo announced a new partnership to 3D-print living human tissues for medical research. Such tissues are intended to provide researchers 'a much more accurate view of how drugs will behave in human beings before those drugs ever enter clinical trials'.

125 In February 2014, Grover *et al* reported having identified a protein able to 'binds with high affinity to all types of human and nonhuman immunoglobulin G' antibodies. The authors note that the protein also blocks antibody-antigen union involving large antigens. These broadly reactive antibody-binding proteins 'have been widely exploited both in the laboratory and in industry for purifying, immobilizing, and detecting antibodies'.

126 In February 2014, Chiappini *et al* reported having developed and use biodegradable silicon nanoneedles to deliver nucleic acids into cells. The authors demonstrated significant progress in overcoming the challenges of delivering genetic material *in vivo* - co-deliver DNA and siRNA with efficiency greater than 90%.

127 A September 2014 review of –omics and imaging technologies on assessing the host immune response to biodefence agents highlighted technological advances which allow the interactions between host and pathogen to be studied in much greater detail. The authors noted progress in next generation sequencing as well as DNA and protein microarrays for 'dissecting the underlying host response to infection at the molecular level'. They noted the utility of flow cytometry and fluorescence microscopy for assessing cellular responses to infection, and biophotonic imaging for visualising the infectious disease process.

128 A July 2012 working paper by China for the BWC Meeting of Experts highlighted 'primary results of human microbiome researches... indicate that our normal physiological functions are closely related to our second genome, whose disorder might affect normal physiological metabolism of humans and even cause illness'.

129 In June 2012, the Human Microbiome Project Consortium published details of the structure, function and diversity of the health human microbiome. This provides a useful normal data set against which disease states can be compared.

130 An April 2013 review of horizontal gene transfer in the human intestine highlighted the potential for the spread of antibiotic resistance genes from commensal organisms to potential pathogens. The authors discussed progress in using culture-independent techniques and metagenomic studies to provided insights into the distribution of mobile genetic elements and the extent of horizontal gene transfer in the human gastrointestinal tract.

131 In October 2013, Chagnot *et al* reported significant progress in characterizing the role of secreted proteins in biofilm formation.

132 In January 2015, Karimi *et al* presented an overview of the role of relevant physical processes in biofilm formation, including propulsion mechanisms, hydrodynamic effects, and transport of quorum sensing signals. The authors also examined opportunities for future microfluids work on biofilms.

133 In August 2012, Houry *et al* reported having using motile bacteria exuding anti-bacterial agents to kill a heterologious biofilm population. Such biofilms can be an important element of the growth of pathogenic bacteria, for example *Staphylococcus aureus*, helping to protect them from antibiotics, hydrodynamic shear and environmental challenges.

134 In January 2015, Sanchez-Vizuete *et al* reported that products from non-pathogenic bacteria were vital for protecting pathogenic strains in biofilms.

135 In April 2013, Savage *et al* reported that biofilm growth of *Staphylococcus aureus* 'dramatically increases horizontal transfer of plasmid-borne antibiotic resistance determinants by conjugation/mobilization and that standard laboratory practices to induce conjugation in staphylococci achieve optimal efficiency owing to the presence of a biofilm'.

136 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'DNA sequencing and genomic microorganisms' certification methods allow in the shortest time possible to carry out the accurate multi-parameter identification of any pathogen, thus playing a key role in investigating the causes and origins of the outbreaks of infectious diseases.

137 In March 2011, Rasko *et al* reported the use of whole-genome sequencing and comparative genomics in a forensic investigation. The authors were able to identify unique phenotypic qualities of an agent, link it to changes in sequence data and then used those sequences to differentiate samples connected with the event from background microbes, including bacteria from the same species and strain.

138 In February 2012, Grad *et al* reported use of whole genome sequencing and other molecular epidemiological approaches to study isolates from outbreaks in France and Germany that were indistinguishable using traditional tests. Similar approaches could provide insights into the origins of an agent, its evolution or reveal a possible disconnect from a natural chain of infection.

139 A July 2012 presentation by Sweden to the BWC Meeting of Experts highlighted recent developments in high-throughput sequencing and bioinformatics, and their potential benefits to the Convention. The author noted the application of these technologies for the detection of covert illegitimate release by helping to identify anomalies in antibiotic resistance, mutational bias, geographic distribution of genetic group, transmission routes, outbreak intensity and dynamics, and clinical manifestations.

140 In June 2012, Sampath *et al* reported a methodology for combining PCR approaches with Electrospray-Ionization Mass Spectrometry to be able to identify agents of concern, differentially from their close relatives.

141 Biodefence specific PCR applications include those offered by BioFire Defense, which according to their own promotional material "develops, manufactures, and sells the fastest, highest-quality machines in the world for real-time detection of pathogens and emerging infection diseases. This technology including DNA amplification, real-time thermocycling and Hi-Res Melting. Our complement of products include the FilmArray and RAZOR EX instruments along with our Hi-Res Melting dyes and kits and our expanding line of freeze-dried reagents and DNA/RNA purification kits".

142 In March 2012, Rodriguez-Lorenzo *et al* reported developing a biosensor for prostate cancer biomarkers over ten times more sensitive than those produced previously. The new device, which can be used in whole serum, makes used of nanoparticles as part of a novel signal-generation mechanism that induces a signal that is larger when the target molecule is less concentrated.

143 In March 2014, Grilli *et al* reported using nano-dispensing for the active accumulation of very diluted biomolecules, enabling easy detection below the fentomolar range. The authors demonstrated being able to detect oligonucleotides down to a few hundreds of attomoles. They used the approach to provide a 60-fold sensitivity improvement of traditional ELISA techniques. The authors envisage developing the approach 'for sensing molecules at very low concentrations, in environmental as well as in diagnostics applications'.

144 In May 2015, Danino *et al* reported hacving designed and built programmable probiotics for detection of cancer in urine. Harmless E. coli were engineered to carry and enzyme which would break down a specific molecule when it was present. The E. coli were then fed to mice where they moved to the liver and began to reside in any liver cancers. When fed the molecule in question, the modified *E.coli* broke it down into other compounds, one of which emits light. That compound makes the urine glow. The strength of the glow is proportional to the amount of bacteria present, which in turn correlates to the amount of cancerous tissue present.

145 An April 2012 review of nanotechnology applications in diagnostics highlighted benefits for simplifying sample preparation, the size of equipment and the speed at which results can be generated. The authors highlighted their use as "bioreceptors and transducers in the diagnosis of infectious diseases in diverse settings" as well as progress in integrating nanomaterials in new approaches, such as surface plasmon spectroscopy, amperometry and magnetic relaxation.

146 In August 2012, Krivitsky *et al* reported using nanowires for on-chip filtering, separation and concentration of blood samples, removing the need for desalination and centrifugation steps in sample preparation.

147 An August 2013 presentation by Poland at the BWC Meeting of Experts highlighted the development of biosensors for Bacillus anthacis. The author notes that the sensor can quantify the concentration of the bacteria as well as detect its presence.

148 In December 2013, Gracie *et al* reported 'a new quantitative assay for detection of three pathogens that result in bacterial meningitis using a combination of lambda exonuclease… and surface enhanced Raman scattering'. The authors demonstrated 'three meningitis pathogens were successfully quantified in a multiplexed test with calculated limits of detection in the pico-molar range, eliminating the need for time consuming culture based methods that are currently used for analysis'.

149 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted 'the combination of sensors with mobile applications and databases (e.g. accessible from a smart phone) may become a powerful method for rapid interpretation of results and access to information on targeted methods of treatment'.

150 In January 2012, Prindle *et al* reported having engineered 'the synchronization of thousands of oscillating colony 'biopixels' over centimetre-length scales through the use of synergistic intercellular coupling involving quorum sensing within a colony and gas-phase redox signaling between colonies'. The authors noted that 'given the repertoire of sensing capabilities of bacteria such as *Escherichia coli*, the ability to coordinate their behavior over large length scales sets the stage for the construction of low cost genetic biosensors that are capable of detecting heavy metals and pathogens in the field'.

151 In January 2013, Handy *et al* reported the discovery of a DNA aptamer that targets Saxitoxin. The authors used a surface plasmon resonance sensor to demonstrate concentration-dependent and selective binding.

152 In March 2013, Meneeley *et al* reported having developed 'a rapid, sensitive, portable and easy-to-use assay' using an innovative planar waveguide device to detect paralytic shellfish toxins in marine algae.

153 In July 2013, Anderson *et al* reported having developed an antibody–quantum dot conjugate for Ricin detection. The authors used fluoroimmunoassays and surface plasmon resonance assays for the sensitive detection of Ricin.

154 In April 2011, Prikutsky *et al* reported using the functional changes of phagocytes during an infection to be able to distinguish between bacterial and viral infections. The authors used a chemiluminescent by-product reaction and data mining algorithms to develop classification models for differentiation. The best model demonstrated 88.9% accuracy in differentiating between viral and bacterial infections in a test involving actual clinical samples. Such a test could help ensure that appropriate therapeutics are used, helping to reduce the use of antibiotics. The authors highlight the potential for developing the approach into a point-of-care test. There have been subsequent developments towards such a point-of-care test, for example, In June 2013, Syed *et al* reported using nanoparticles to be able to significantly reduce the physical space needed for chemiluminescent studies, which 'may be readily adapted for developing various miniaturized multiplex biosensors for rapid chemical/biochemical analyses'.

155 A July 2012 presentation by Japan to the BWC Meeting of Experts highlighted the dual use potential of recent developments, including nanobiology 'enabling detection of disease-causing or toxic agent(s) via nanobiosensors'.

156 A July 2012 working paper by Australia for the BWC Meeting of Experts highlighted 'convergence of chemistry and biology (and related aspects of nanotechnology) for the BWC, including developments in detection of pathogens and toxins (biosensors), medical countermeasures, decontamination, and laboratory analysis and identification techniques, including bioforensics. However, it is also recognised that these developments could potentially be misused, including an increased potential for misuse of toxins and bioregulators for hostile purposes'.

157 An August 2013 review of artificial spores highlighted that 'robust cell-in-shell structures have displayed unprecedented characteristics, which include the retardation of cell division and extensive cytoprotective capabilities that encompass exposure to osmotic pressure, shear force, heat, UV radiation, and lytic enzymes. Additionally, the nanothin shells act as highly versatile scaffolds for chemical functionalization to equip cells for implementation in tissue engineering, biosensors, cell therapy, or other biotechnological applications'.

158 A June 2014 review of the implications of the convergence of biology and chemistry highlighted 'nanoparticles are also finding applications in sensor and detection technologies'. The authors noted 'recent trends… towards miniaturised disposable devices, many constructed with nanosized materials for transduction, and as a support for the sensing element'.

159 In June 2013, Lu *et al* reported the potential to use synthetic biology to develop bacteriophage-based 'near-real-time microbial diagnostics… for food, clinical, industrial, and other environmental settings'. These concepts have subsequently been commercialised and Sample6 Technologies offers 'the first in-plant, in-shift pathogen detection for listeria, approved by the USDA & AOAC' intended to 'help shift food safety from reaction to prevention'.

160 A March 2013 review of DNA origami highlighted the use of DNA nanostructures to create 'spatially organized enhanced enzymic cascades'. These were then used to improve the efficiency of bioassays for use in medical diagnostics and environmental modeling. The spatial control of enzymic cascades is also believed to influence the rate of catalysis, with potential to offer benefits for increased yields.

161 In May 2013, Hirtz *et al* reported an improved way to make use of the graphene surfaces used in biosensors. The authors replaced the traditional silicon dioxide substrate used to attach functional elements with a phospholipid membrane which spread more evenly. They also used a different application methodology enabling the multiplexing of membranes, allowing 'different functionalities in close proximity to each other'.

162 In April 2014, Krejcova *et al* reported using two different cadmium quantum dots for the sub-typing and quantification of hemagglutinin.

163 In February 2014, Kotula *et al* reported having designed and built Programmable bacteria detect and record an environmental signal in the mammalian gut. The bacteria, a harmless E. coli, had two devices added – a bi-stable switch and a memory unit. In the presence of a specific antibiotic, an inserted gene transcribed which in turn causes a conformational change in the memory device, which remains in that state for many cell divisions. The authors demonstrated that the sensor and memory system work in mice treated with the antibiotic. They then moved the system into another E. coli strain naturally found in the guts of mice.

164 In May 2011, Bendall *et al* reported having 'used single-cell "mass cytometry" to examine healthy human bone marrow, measuring 34 parameters simultaneously in single cells'. The authors monitored the 'signalling behaviour of cell subsets' using '18 simultaneous markers of functional signalling states' whilst the system was 'perturbed by a set of ex vivo stimuli and inhibitors'.

165 In May 2012, Dove *et al* reported having identified a number of protein changes in lung cells infected with H1N1 influenza virus. The authors note the potential to detect the protein changes as a diagnostic marker.

166 In June 2015, Xu *et al* reported a method for comprehensive analysis of antiviral antibodies in human sera. The authors assert that a 'complete history of viral exposure over a lifetime can be deduced from a drop of blood'. They demonstrated the approaches effectiveness for determining viral exposure and characterizing viral B cell epitopes in high throughput and at high resolution. The potential for clinical application has been noted.

167 In December 2012, Kwong *et al* reported having developed a range of synthetic biomarkers for diseases, including liver fibrosis and cancer. The biomarkers are comprised of peptides joined to nanoparticles, capable and are detectable in urine using multiplexed mass spectrometry. They used their approach to detect relevant conditions in a mouse model and reported effective monitoring "without the need for invasive core biopsies" and improved early detection when compared with current clinically used blood biomarkers.

168 In March 2014, Warren *et al* reported having "designed nanoscale agents that are administered to reveal the presence of diseased tissues by producing a biomarker in the urine that can be detected using paper strips similar to a home pregnancy test". The authors demonstrated in a mouse model that it is possible to "detect diseases as diverse as solid cancer and blood clots using only a single injection of our diagnostic followed by urine analysis on paper". They noted such approaches do "not require expensive instruments, invasive procedures, or trained medical personnel, and may allow low-cost diagnosis of diseases at the point-of-care in resource-limited settings".

169 In October 2014, Warren *et al* reported built upon earlier work to produce nanoparticle-based biomarkers for non-communicable diseases which can be detected in urine. The authors reported the development of novel detection assays enabling the diagnosis of a new disease.

170 In October 2011, Tran *et al* reported a top-down approach to identifying gene products in human cells. The authors used 'a new four-dimensional separation system, identification of 1,043 gene products from human cells that are dispersed into more than 3,000 protein species created by post-translational modification (PTM), RNA splicing and proteolysis'. The authors noted a 'greater than 20-fold increases in both separation power and proteome coverage, enabling the identification of proteins up to 105 kDa and those with up to 11 transmembrane helices'. The asserted that this 'technology promises to improve the link between proteomics data and complex phenotypes in basic biology and disease research'.

171 In January 2013 two papers were published detailing preclinical genetic signatures for different human infections. Woods *et al* reported a gene signature for H1N1 influenza present in 94% of infected cases, detectable as soon as 29 hours post infection and reaching maximal accuracy after 43 hours. The authors reported using the approach to be able to distinguish patients in an Emergency Department infected with H1N1 influenza with 92% accuracy. Ahn *et al* reported using the gene expression associated with host inflammatory responses to differentiate between S. aureus and E. coli infections in humans and mice.

172 A 2013 review of nanotechnology applications in public health highlighted approaches for improved measuring and perturbing of living systems. The authors concluded that nanotechnology can be used to characterize single molecules or cells at extraordinarily high throughput and deliver therapeutic payloads to specific locations as well as "to exhibit dynamic biomimetic behavior". Resulting tools had produced improvements in understanding disease, especially in terms of systems biology, as well as novel therapeutic strategies.

173 An April 2013 review of clinical applications of molecular diagnostics examined genetic testing to identify clinical conditions and to influence the treatment course. The authors highlighted clinical testing by sequencing the coding regions of the genome but noted the scale of bioinformatic analysis required. The authors also addressed testing for genetic components that influence drug metabolism or interaction of a drug with its cellular target.

174 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted a number of developments relevant to biomarkers, including:

- RNA-seq, using next generation sequencing technology which can 'be conducted without a priori knowledge of the transcript's sequence' and 'may be used to determine comparative gene expression data'
- It is now possible 'to simultaneously determine the pathogen as well as the host's gene expression profile during infection'; and
- 'This technology may be utilised for the early diagnosis of exposure to chemical or biological weapons, based on the expression patterns of a set of predetermined biomarker genes'.

175 In January 2014, Siddle *et al* reported having used genome-wide expression profiling revealed that ~40% of miRNAs are differentially expressed upon infection *Mycobacterium tuberculosis*. The authors identified two infection-specific response expression quantitative trait loci.

176 In September 2013, Zaas *et al* reported having used a reverse transcription polymerase chain reaction (RT-PCR) TaqMan low-density array (TLDA) platform to classify respiratory viral infection. The authors demonstrated that the host gene expression signatures they had developed for H3N2 and H1N1 influenza infections could be used to classify infections in 102 individuals presenting at an emergency room. They reported a sensitivity of 89% and a specificity of 94%.

177 A November 2013 review of emerging nanotechnology-based tools for biology and medicine, highlighted the potential to use nanostructure for isolating rare biomarkers from the complex and heterogeneous mixtures of proteins. The authors note that their 'high surface areas can be used to capture clinically relevant biomarkers through molecular recognition processes. The enhanced chemical and physical properties can be then be used to detect or isolate these biomarkers'. They provide two examples where such approaches have enabled multiplexed readout of up to 12 separate biomarkers with high dynamic ranges. The authors also reviewed approaches for 'amplifying the initial signal, increasing sensitivity using stable synthetic peptides that do not occur biologically (low background), and multiplexing'.

178 A January 2013 review of the use of Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for detection of antibiotic resistance mechanisms in pathogens. The authors noted "MALDI-TOF MS has been successfully applied as an identification procedure in clinical microbiology and has been widely used in routine laboratory practice because of its economical and diagnostic benefits". Recognising that MALDI-TOF has become a common diagnostic tool, the authors examined developments in "the detection of antibiotic modifications by degrading enzymes, the detection of resistance mechanism determinants through proteomic studies of multiresistant bacteria, and the analysis of modifications of target sites, such as ribosomal methylation".

179 In October 2013, Jeng *et al* reported using reverse transcription PCR and Electrospray ionization mass spectrometry to detect a range of relevant pathogens in human respiratory samples in 8 hours. The authors were detecting *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Brucella* spp., *Burkholderia spp*., and *Rickettsia prowazekii*. They reported 73.6% specificity and 81.8% sensitivity for bacterial pathogens, 97.3% specificity and 93.3% sensitivity for viral pathogens, and 97.8% specificity and 42.6% sensitivity for fungal pathogens.

180 In January 2015, Filipiak *et al* reported having used gas chromatography-mass spectrometry to detect pathogens in the lower respiratory tract of ventilated patients. The authors concluded the "study provides proof of the concept that the appearance and the concentration profile of pathogen-derived metabolites… in the breath of ventilated patients… correlates with the presence of a particular pathogen".

181 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted:

- 'Recently developed ionization methodologies for high-resolution MS that enable analysis with minimal sample preparation. These methods are appropriate for analysis of complex compounds at trace concentration levels in complex matrices, such as biological samples'; and
- 'Work using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) that uses a so-called "soft" ionization method that enables the direct analysis of large biological molecules'.

182 A May/June 2013 review of Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for profiling of bacteria at the strain level highlights that strain categorization, strain differentiation, and strain identification have been enabled through the use of library-based and bioinformatics-enabled approaches. Current challenges are also noted including that "library-based approaches can be limited by effects of sample preparation and culture conditions on reproducibility, whereas bioinformatics-enabled approaches are typically limited to bacteria, for which genetic and/or proteomic data are available".

183 In November 2013, Branca *et al* reported 'a liquid chromatography–mass spectrometry (LC-MS)-based method permitting unbiased… genome-wide discovery of protein-coding loci in higher eukaryotes'. The authors demonstrated having 'probed the six-reading-frame translation of the human and mouse genomes and identified 98 and 52 previously undiscovered protein-coding loci, respectively. The method also enabled deep proteome coverage, identifying 13,078 human and 10,637 mouse proteins'.

184 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted:

- 'The miniaturisation of various MS [mass spectrometry] instruments and the development of field-amenable MS sources (sample introduction and ionisation interfaces';
- 'Smaller molecules are readily made volatile and analysed by gas chromatography mass spectrometry (GC-MS)';
- 'The advent of electrospray ionisation (ESI) and matrix assisted laser desorbtion/ionization (MALDI) ion sources' increased the number and range of biological molecules that can be analysed;
- 'Methods of sample introduction (without pre-preparation) continue to evolve with the development of techniques such as Matrix Assisted Ionisation Vacuum (MAIV) and Desorption Electrospray Ionisation (DESI)mass spectrometry'; and
- 'Time of flight (TOF) seems most amenable to biological analyses as it is not as limited by analyte molecular weight; also, the technology may be made field portable and is compatible with ionisation techniques'.

185 In February 2011, Comstock *et al* reported having combined fluorescent microscopy techniques (which 'allow researchers to observe proteins as they conform and move, but often lack the spatial range to track the protein's motion over distance) with optical traps (which 'enable researchers to study a protein's translocation, but not its conformation').

186 In May 2011, Planchon *et al* reported having used scanned Bessel beams in conjunction with structured illumination and/or two-photon excitation for three-dimensional (3D) subcellular imaging. The authors used this approach to image 'mitochondria, filopodia, membrane ruffles, intracellular vesicles and mitotic chromosomes in live cells'.

187 A July 2015 review of 'frugal science' highlighted the development of cheap, paper based microscopes. The author noted that both lower and higher resolution versions had been developed and cost $0.5 and $1 respectively. They also highlighted the existence of disease specific versions, for example for malaria, to aid diagnostics in resource limited settings.

188 In February 2013, Ramachandraiah *et al* reported converting a commercial DVD drive into a laser-scanning microscope that can analyse blood and perform cellular imaging with one-micrometre resolution.

189 In February 2012, Iverson *et al* reported a novel approach to reconstructing genomes from fragments captured during metagenomic analysis. The authors demonstrated the ability of their approach by identifying as-yet uncultured marine group II Euryarchaeota from a seawater sample. The authors note that this approach 'can overcome assembly difficulties caused by interstrain variation in complex microbial communities, enabling inference of ecosystem functions for uncultured members'.

190 In August 2012, Tse *et al* reported using high-throughput sequencing and metagenomics approaches of rectal swabs from bats, dogs, cats, and monkeys to identify a previously unidentified papillomavirus. An ability to identify previously unknown pathogens increases the range of potential weapons agents.

191 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted that 'often times, the causative agent of disease cannot be determined using techniques such as PCR. In these instances a shotgun metagenomic approach has led to the identification of novel pathogens as well as a number of unknown genetic sequences'.

192 A July 2012 presentation by Mexico to the BWC Meeting of Experts highlighted the use of metagenomics for the identification of organisms present in a mixed environmental sample. They noted its use in identifying and tracking a specific agent. The author provided an example of the use of metagenomics approaches to study the cerebrospinal fluid of patients with meningoencephalitis.

193 A July 2012 presentation by Sweden to the BWC Meeting of Experts highlighted recent developments in high-throughput sequencing and bioinformatics, and their potential benefits to the Convention. The author noted the application of these technologies for bioforensics, including:

- Providing background data, such as agent population structure data, genotype distribution data, and high-resolution matching with possible sources; and
- Insights into production methods, such as particle sizes, associated signatures, geographical origins, and intelligence information.

194 In July 2014, Budowle *et al* reported having developed criteria that should be considered for validating high-throughput sequencing technologies for use in microbial forensics. The authors detail how performance and limitations of such platforms, including analytical processes, assays and data interpretation, can impact the interpretation of results, influencing their reliability for security-related investigations.

195 In July 2012, Inouye *et al* reported a software tool that enables whole genome studies involving bacterial pathogens to correlate their results against multi-locus sequence typing databases – the former gold standard for genetic identification of bacterial species. This backwards compatibility provides historical context for ongoing studies.

196 In August 2012, van Boheemen *et al* reported for a novel coronavirus the complete genome sequence, genome organization, expression strategy and relationship with known viruses. The authors determined that the new virus (ultimately responsible for MERS-CoV) was closely related to a bat virus but was distant from the causative agent of SARS). Similar approaches could help determine the origin and evolutionary dynamics of an agent used in a deliberate attack. The authors conclude that the results 'will be vital to rapid advancement of both clinical and vital research on this emerging pathogen'.

197 In January 2013, Yap *et al* reported data on the persistence of bacterial genomic DNA following autoclaving. They highlighted the potential for fragments of genetic material to be sufficiently complete to pose a risk for horizontal gene transfer – for bacterial species that come into contact with it to pick it up and to develop new properties. The authors highlight the potential for virulence genes, environmental persistence, or antibiotic resistance characteristics to spread in this manner. The results also offer interesting possibilities for downstream sampling (either in time or location) of effluent from facilities enabling inferences to be made as to what agents are being used, what characteristics those agents posses, and what activities were being undertaken. Equally, similar techniques might offer opportunities to find usable bioforensic data even after equipment has been autoclaved.

198 In April 2011, Mak *et al* reported using real-time reverse–transcription PCR–based genotyping for rapid and simple differentiation of H1N1 influenza reassortants.

199 A July 2012 presentation by Sweden to the BWC Meeting of Experts highlighted recent developments in high-throughput sequencing and bioinformatics, and their potential benefits to the Convention. The author noted the application of these technologies for laboratory identification for treatment and prophylaxis, including the type of infectious agent, its genus and species, antimicrobial resistance, and contagiousness. The author also highlighted how these technologies had been used during the German E. coli outbreak 2011 and an endemic tularaemia outbreak in Sweden, to provide a probable genomic history of the outbreak strain.

200 In April 2013, Loman *et al* reported using culture-independent sequence-based metagenomics approaches to investigating an outbreak of E. coli. The authors demonstrated that metagenomics approaches could be used to identify pathogens during an outbreak of diarrheal disease without the need to culture the pathogen. They noted 'challenges include improving diagnostic sensitivity, speeding up and simplifying workflows, and reducing costs'.

201 In September 2012, Chan *et al* reported having used whole genome sequencing of field isolates of Plasmodium vivax, the pathogen responsible for malaria. They demonstrate the feasibility of using such approaches to characterise the genetic diversity of the parasite and to better understand drug resistance, and erythrocyte invasion, all of which has been hampered by an inability to culture the pathogen in a laboratory. Comprehensive, characterised sequence data could potentially enable the synthesis of pathogens impossible or difficult to culture from the wild.

202 A January 2013 review of the use of sequencing in the management of *Staphylococcus aureus* infections highlighted the importance of whole genome sequencing which 'could be used to predict resistance, assess virulence, and type isolates at the highest possible resolution. The results generated could be used to guide clinical management and infection control practice. Studies using bench-top sequencing machines have already demonstrated the feasibility of such approaches'.

203 In August 2013, Albariño *et al* reported having used whole genome sequencing to confirm the causative organisms and order of emergence of four viral hemorrhagic fever outbreaks in Uganda and the Democratic Republic of the Congo in 2012. The authors noted that 'sequence analyses provided a tool to confirm infection and transmission events'.

204 An August 2013 presentation by Poland at the BWC Meeting of Experts highlighted the use of pyrosequencing to detect 'short DNA stretches in real-time using biotinylated PCR amplicons'. The authors described using this approach to detect virulence plasmids associated with *Bacillus anthracis* 'in food matrices including milk, juice, bottled water, and processed meat'.

205 A July 2012 presentation by Germany to the BWC Meeting of Experts highlighted the use of nucleic acid- based techniques for diagnosis. The authors highlighted DNA Analysis and PCR screening of clinical samples, DNA sequencing and synthesis, as well as bioinformatics challenges, which the author described as 'the bottleneck of modern approaches'

206 A June 2013 review of data from sequencing highlighted that 'DNA sequencing is on the path to becoming an everyday tool in life-science research and medicine. Institutions such as the Mayo Clinic and the New York Genome Center are beginning to sequence patients' genomes in order to customize care according to their genetics'. The authors noted that 'computing, not sequencing, is now the slower and more costly aspect of genomics research… Sequencers are improving at a faster rate than computers are'. The authors highlighted a number of recent efforts to address this challenge, including 'both better algorithms and a renewed focus on such "big data" approaches as parallelization, distributed data storage, fault tolerance, and economies of scale'. They noted the utility of text compression to create algorithms that can better package genomic data and cloud computing models for searching through the information'.

207 An August 2013 working paper submitted by the United States of America to the BWC Meeting of Experts highlighted 'High-throughput DNA sequencing is becoming faster and less expensive – and hence more frequently used for identifying unknown pathogens, outbreak sources and animal reservoirs. Parallel advances in computational biology are accelerating the analysis of enormous databases generated by sequencing technologies'.

208 In September 2013, Li and Chen reported having used sequence information to develop a 'hypothesis of the evolutionary history of Ebola virus'.

209 In June 2014, Naccache *et al* reported a novel bioinformatics platform that overcomes the 'challenge of analyzing results accurately and in a clinically relevant timeframe' for the use of genome sequencing. The authors demonstrated 'In fast mode, SURPI detects viruses and bacteria by scanning data sets of 7 – 500 million reads in 11 min to 5 h, while in comprehensive mode, all known microorganisms are identified, followed by de novo assembly and protein homology searches for divergent viruses in 50 min to 16 h.'.

210 A November 2013 review of emerging nanotechnology-based tools for biology and medicine, highlighted the potential to use nanopores to split and sequence DNA. The authors noted the challenges of achieving 'single-base-pair resolution due to the stochastic motion of DNA as well as the measurement sensitivity at fast translocation speeds'.

211 In August 2011, Deng *et al* reported having used pyrosequencing for the rapid detection and sub-typing of human influenza a viruses and reassortants. The authors reported 'all eight gene segments of 57 laboratory isolates and 17 original specimens of seasonal H1N1, H3N2 and 2009 H1N1 pandemic viruses were correctly matched with their corresponding subtypes. In addition, this method was shown to be capable of detecting reassortant viruses by correctly identifying the source of all 8 gene segments from three vaccine production reassortant viruses and three H1N2 viruses'.

212 A July 2012 presentation by Spain to the BWC Meeting of Experts highlighted molecular methods to monitor plant rhizobacterial communities. The author noted a number of relevant technologies including: PCR as being highly specific and cost-effective; microarray hybridisation as useful for continuous monitoring at a moderate cost; and genome sequencing as comprehensive, being capable of taxonomically analysing all rhizobacterial components and estimating biodiversity.

213 A July 2012 working paper by China for the BWC Meeting of Experts highlighted 'revelation of pathogenic microbes' genome evolution and its relation with infectivity and pathogenicity and greatly enhances the surveillance, diagnosis and therapy of related infectious diseases. There is no doubt that such DNA sequence information can also be used for the modification of antigenicity, infectivity, toxicity and drug resistance of traditional pathogens, even for the artificial design and synthesis of totally new pathogens, which will lead to the failure of traditional prevention and treatment of infectious diseases and make efficient prevention and control more difficult'.

214 In April 2013, Relman published an overview of the use of sequencing in identifying and investigating disease outbreaks. He noted that rapid sequencing of the pathogen's genome 'provides a wealth of information about the functional potential of the organism' and that metagonomic sequencing (simultaneously sequencing fragments from the full range of organisms captured from a particular environment) 'provides insights into the potential activities of a microbial community, possible interactions between microbial community members, and the nature of their relationships with their environment (e.g. a human host)'.

215 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted that next generation sequencing 'may be utilised to sequence the 16S ribosomal RNA gene (16S rRNA) which would give an indication of the identity of the members of a mixed bacterial community in a sputum sample'.

216 In January 2012, Calvo *et al* reported having used next-generation sequencing to diagnoses cases of Infantile Mitochondrial Disease. The authors sequenced '42 unrelated infants with clinical and biochemical evidence of mitochondrial oxidative phosphorylation disease'. They showed that '23 of 42 (55%) patients harboured… recessive genes or pathogenic mtDNA variants. Firm diagnoses were enabled in 10 patients (24%) who had mutations in genes previously linked to disease. Thirteen patients (31%) had mutations in nuclear genes not previously linked to disease. The pathogenicity of two such genes, NDUFB3 and AGK, was supported by complementation studies and evidence from multiple patients, respectively'.

217 In December 2014, Muniraju *et al* reported the use of whole and partial genome sequencing to produce a phylogentypic map of all 4 strains of peste des petits ruminants virus. The authors use the data to investigate evolutionary and epidemiologic dynamics of the virus, identifying common ancestors and the physical locations where divergence of strains began.

218 In December 2014, Ward *et al* reported a phylogenetic analysis of a series of *Staphylococcus aureus* bacteria responsible for infections in the United Kingdom. The authors mapped traits onto phylogenies, focusing in particular on a specific antibiotic resistance factor. Specific human and animal related clades were identified and the relationship between livestock and hospital transmission explored. The researchers also reported significant differences in the gain and loss, as well as dynamics of resistance to methicillin and tetracycline related to contrasting historical patterns.

219 In a May 2014 review, Spackman noted '[c]ompared to serology both RT-PCR and sequencing are preferred sub-typing methods because of the number of reference reagents which need to be prepared for serological methods and results of molecular methods are often easier to interpret'.

220 In September 2014, Bacconi *et al* reported sensitivity and selectivity data for an integrated sample preparation, PCR platform able to identify a range of pathogens. The authors report that the platform can process whole blood samples and makes use of PCR followed by electrospray ionization mass spectrometry. Whilst less sensitive than culturing the causative organisms, the authors conclude that such platforms do have "the potential to provide rapid detection and identification of organisms responsible for bloodstream infections".

221 In January 2014, Nhu *et al* reported on the use of the GenXpert PCR-based platform for the clinical diagnosis of and detection of a specific antimicrobial resistance factor in Tuberculous meningitis during an outbreak in Vietnam. The GenXpert test "is a closed-cartridge-based system that is easy to operate by minimally trained staff and gives results in approximately 2h". The authors reported high levels of selectivity and sensitivity levels comparable to traditional testing methodologies in non-specialist laboratories but with increased levels of throughput.

222 In September 2015, Liljander *et al* reported having developed a field-applicable test for an important disease in goats. The authors used rapid, specific, and sensitive assay employing isothermal DNA amplification using recombinase polymerase amplification. The platform was powered by a car battery and used clinical samples from goats (with minimal processes or DNA extraction) to provide a signal output in 15 – 20 minutes. The authors noted that existing diagnostic approaches involved "cultivation, serological assays, and PCR, are time-consuming and require fully equipped stationary laboratories, which make them incompatible with testing in the resource-poor settings that are most relevant to this disease".

223 In June 2012, Kumar *et al* reported A multiplex PCR assay for the simultaneous identification of virulent and avirulent *Bacillus anthracis*.

224 In March 2013, Wu *et al* reported a multiplex reverse transcription-PCR assay for the detection of influenza A virus and differentiation of the H1, H3, H5 and H9 subtypes. The successfully used the assay for virus identification and differentiation in human and avian clinical samples.

225 A May 2015 review of PCR techniques for vaccine development highlighted progress in 'virus quantification to optimize conditions in cell culture or in the associated downstream purification steps'. The authors describe how Digital droplet PCR separates and isolate single molecules which are then amplified. 'The droplet's fluorescent intensity depends on the presence or absence of the target; as such, positive and negative droplets are identified, which allows for absolute quantification of the viral genomes'. The authors assert that such an approach offers advantages:

- Negating need for a standard;
- The extracted RNA does not need to be purified from the reagents needed to lyse the virus;
- Viral associated RNA released by infected cells can be measured directly; and
- Potential for duplexing with a second assay that measures host cell DNA concentration.

226 In September 2012, Chiapponi *et al* reported developing a Multiplex RT-PCR assay for differentiating European swine influenza virus subtypes H1N1, H1N2 and H3N2. The authors demonstrated 100% specificity for isolated viruses, and 89% specificity from samples where no viral isolation had been attempted. They used the test to 'identify mixed viral infections and the circulation of a reassortant strain before performing genomic studies'.

227 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted:

- 'An array-based real-time PCR system that combines sample preparation, PCR amplification and data analysis onto a single instrument with very little user involvement';
- 'Loop mediated isothermal amplification (LAMP) technology' for DNA extraction and amplification for detection of agents;
- An isothermal-based 'system that does not require electricity (socket or battery) to perform Clostridium difficile diagnostics in extremely resource limited settings'.

228 An August 2013 working paper submitted by the United States of America to the BWC Meeting of Experts highlighted 'Recent advances in molecular assays include the application of "isothermal amplification" of nucleic acids; these assays can be performed without thermal cycling equipment while providing essentially the same information as more expensive polymerase chain reaction (PCR) testing'.

229 An August 2013 working paper submitted by the United States of America to the BWC Meeting of Experts highlighted 'For some infectious diseases, generally inexpensive POC [point-of-care] devices are available that can increase the speed and accuracy of diagnosis.... Recent advances in technology benefit diagnosis... at POC... by providing more rapid identification and more precise information about known and, in some cases, newly emerged pathogens'. The authors identified a 'trend in recent years... to make sophisticated tests, or assays, more easily performed with less training, leading to decentralization and diagnosis closer to the POC'.

230 A May 2015 review of point-of-care diagnostics noted:

- There is worldwide interest in developing point-of-care diagnostics, with 110 developing products in every region of the world.
- The market is highly granular, and different disease classes have different, characters and make different contributions.

- They are particularly useful in combating high-impact disease where rapid diagnostics and treatment is critical.
- Their primary use is in dealing with infectious disease (70% of point-of-care marketplace).
- Applications have been found for anthrax, ricin and bio-warfare agents and whilst the amount of products available is increasing, point-of-care diagnostics associated with these diseases still represent only a fraction of the market.
- The use of point-of-care diagnostics for optimization of drugs and medication is an emerging theme.
- PCR amplification is increasingly common in point-of-care diagnostics.
- A wide range of specific biomakers are 'an almost unexplored space' and that only a few genetic markers with known relevance for diagnostics and therapeutics are referenced regularly suggesting that with a few exceptions this was 'still virgin territory, vis-à-vis molecular biomarkers'.

231 In November 2014, Pardee *et al* reported having developed paper-based synthetic gene networks. The authors freeze dried commercially available cell-free systems onto paper and demonstrated an ability to integrate small-molecule and RNA actuation of genetic switches, rapid prototyping of complex gene circuits, and programmable in vitro diagnostics. They envisage "inexpensive, sterile, and abiotic distribution of synthetic-biology-based technologies for the clinic, global health, industry, research, and education". The authors described having built paper-based genetic circuits with "colorimetric outputs for detection by eye" for use in the field and fabricated "a low-cost, electronic optical interface". They constructed glucose sensors and strain-specific Ebola virus sensors.

232 Commercial point-of-care devices have begun to be integrated with smart phones. For example the ApolloDX, according to their own promotional material, uses the such devices for processing power and connectivity to "provide laboratory-quality test results in 10 minutes or less, expediting diagnosis and treatment for patients".

233 In May 2012, Japanese researchers reported having produced a rapid, point-of-care test for all strains of H5N1 influenza, significantly improving the range of in field detection. In June 2013, researchers from the same institution reported developing 'a biochip that can identify 13 different major tropical diseases—including dengue fever, chikungunya, hand, foot and mouth disease, and malaria—from a single blood sample'.

234 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted:

- 'Various advances have been made in the fields of microfluidics and nanotechnology which may aid the point-of-care diagnosis of TB and other diseases. A variety of mechanical, biochemical and electrical detection methods are in development for the detection of this organism';
- 'Mass/Piezoelectric detectors' which can detect pathogens in real time';
- Field portable versions of classical ELISA (Enzyme Linked Immunosorbancy Assay) using lateral flow chromatography;
- 'An array-based real-time PCR system that combines sample preparation, PCR amplification and data analysis onto a single instrument with very little user involvement';
- 'Loop mediated isothermal amplification (LAMP) technology' for DNA extraction and amplification for detection of agents;
- An isothermal-based 'system that does not require electricity (socket or battery) to perform Clostridium difficile diagnostics in extremely resource limited settings'.

235 In August 2015, Cabibbe *et al* reported efficacy data for the use of an integrated lab-on-a-chip platform for the diagnosis of a multi-drug resistant pathogen. The authors reported high specificity and sensitivity and almost 98% efficacy when compared to whole genome sequencing but with significant time savings. The authors concluded that lab-on-a-chip platforms are "promising tools to fill the diagnostic gap in low-income countries: they integrate many of the laboratory components on a small chip, thus reducing infrastructure and technical requirements but preserving analytical capabilities". The authors also noted benefits in terms of "operating speed, ease of modification (addition/removal of probes), the ability to perform multiplex tests and to scale-down costs". (to add to C(v) Distributed diagnostics.

236 A May 2011 review of the advances in microfluidics to point lab-on-a-chip technologies highlighted 'a large number of publications and patents of microfluidic devices functioning as pumps, mixers, concentrators, and valves, which are the building blocks for creating functional bioreactors and lab-on-a-chip systems'. The noted recent progress in: capillary driven and paper-based microfluidics; multilayer soft lithography; multiphase microfluidics; electrowetting-on-dielectric driven droplet microfluidics; electrokinetics; and centrifugal microfluidics.

237 A January 2013 review highlighted the development of bio-based polymer beads use in point-of-care and lateral flow devices. The author reports that these beads are cheaper to produce, more environmentally friendly, and multi-functional – capable of attaching multiple proteins onto a single bead. The company has since expanded its product range to use biobeads for antigen and vaccine delivery, biocatalysis, bioseparation, and diagnostics. A spin off company is 'intended to commercialize it core bionanoparticle technology for cancer therapeutics and autoimune diseases'.

238 A November 2013 review of emerging nanotechnology-based tools for biology and medicine, highlighted the potential of integrating nanomaterials and microfluidics for application in point-of-care diagnostics and implantable monitoring devices. The authors noted that 'microfluidic technologies with integrated sensors for processing bodily fluids such as blood or saliva can be manufactured very reliably in a high-throughput format and also are very robust'.

239 An August 2013 working paper submitted by the United States of America to the BWC Meeting of Experts highlighted 'more advanced (and likely more expensive) diagnostic tests may require specialized equipment, resources and laboratories (e.g. reference laboratories) but can provide more detailed information about pathogens. Recent advances in technology benefit diagnosis… by providing more rapid identification and more precise information about known and, in some cases, newly emerged pathogens'. The authors note a trend towards the 'consolidation of testing in larger labs, driven by economies of scale, availability of expertise, and the development of newer diagnostic approaches that have substantial complexity and costs'.

240 In April 2013, Zhang *et al* published a review of strengths and weaknesses of disease surveillance and notification systems in light of the 2009 H1N1 influenza outbreak. Their review concludes that 'investments in global surveillance and notification systems made an important difference in the 2009 H1N1 pandemic. In particular, enhanced laboratory capacity… led to earlier detection and characterization of the 2009 H1N1… In addition, improved global notification systems contributed by helping health officials understand the relevance and importance of their own information'.

241 A June 2014 review of the implications of the convergence of biology and chemistry highlighted 'the natural metabolic pathways have been published for the production of Saxitoxin, ricin and many other toxins. In vitro biosynthesis of Ricin and Saxitoxin has been described'. The authors noted 'at present, obtaining ricin, and the small quantities of Saxitoxin required for permitted purposes… from their natural sources is simpler than employing metabolic engineering strategies. It was however noted that as enabling technologies become less expensive, more efficient, and more available, this assessment may need revising'.

242 In October 2011 Negredo *et al* described the discovery of a new filovirus responsible for bat deaths in Spain. Previously, filoviruses, which cause diseases such as Ebola Virus Disease and Marburg disease, had been believe to be confined sub-Saharan Africa. This paper demonstrates that pathogens relevant to the BWC might be found in a wider range of environments than previously thought.

243 In August 2012, Snitkin *et al* reported using Whole Genome Sequencing to track a hospital outbreak of multi-drug resistant pathogen in real time. Having integrated genomics approaches with epidemiological analysis, the authors were able to trace the origins of the outbreak, to identify unexpected transmission routes and to provide possible explanations for these transmissions. The concluded that such approaches offer important 'actionable insights and facilitate the control' of infections.

244 In April 2014, Wagner *et al* reported have compiled draft genomes from individuals to have died from *Yersinia pestis* during the first recorded plague pandemic, the Plague of Justinian (6 – 8th centuries). The authors used phylogenic analysis to demonstrate that the responsible Y. pestis strain represented a novel branch which 'has no known contemporary representatives, and thus is either extinct or unsampled in wild rodent reservoirs'.

245 In January 2013, Holden *et al* reported having used a variety of genomics approaches to track the emergence, evolution and global spread of methicillin resistant *Staphylococcus aureus*. The report having identified the molecular basis of 99.8% of antimicrobial resistance phenotypes and having documented the genetic changes associated with adaptation to the hospital environment and with increasing drug resistance over time.

246 In October 2011, Bos *et al* reported 'a reconstructed ancient genome of *Yersinia pestis* at 30-fold average coverage from Black Death victims securely dated to episodes of pestilence-associated mortality in London, England, 1348 – 1350'. The authors connected the sequence data to other known historical and contemporary strains. They demonstrated that 'no unique derived positions in the medieval organism, indicating that the perceived increased virulence of the disease during the Black Death may not have been due to bacterial phenotype. These findings support the notion that factors other than microbial genetics, such as environment, vector dynamics and host susceptibility, should be at the forefront of epidemiological discussions regarding emerging Y. pestis infections'.

247 In August 2012, Coombs *et al* reported using a raft of molecular biology techniques to study the genetic relatedness of a strain of *Staphylococcus aureus* isolated throughout different parts of Australia over an extended period. The were able to determine that there was very little genetic diversity amongst isolates, that core regions of the genome are very stable and that the strain independently acquired key elements in different physical locations.

248 In August 2015, Carroll *et al* reported deep sequencing of Ebola Virus from 179 patients from the outbreak in West Africa to trace the genetic evolution of the virus and the emergence of different lineages. The data generated was used for metabolic epidemiological analysis. The authors noted such an approach "can be used in conjunction with epidemiological information to test retrospectively the effectiveness of control measures, and provides an unprecedented window into the evolution of an ongoing viral haemorrhagic fever outbreak".

249 In February 2013, Hay *et al* published a review evaluating 'the state of knowledge of the geographical distribution of all infectious diseases of clinical significance to humans'. They examined both data availability and methods used in mapping processes. They determined that mapping about half the relevant diseases was possible but that only 4% has been comprehensively mapped. They also identified a number of factors hindering such efforts and the importance of embracing non-conventional data sources to overcome them. Such mapping would provide important normal data facilitating the identification of both natural and deliberate disease events.

250 An August 2013 working paper submitted by South Africa to the BWC Meeting of Experts highlighted 'An isothermal-based 'system that does not require electricity (socket or battery) to perform Clostridium difficile diagnostics in extremely resource limited settings'. The author noted 'the researchers used a solid phase extraction manifold to isolate DNA under vacuum, which was created using a standard bicycle pump. Target DNA amplification was achieved isothermally on a microfluidic chip in a simplified instrument that used commercially available "toe warmers" to maintain the reaction temperature over the required time period. The chemical reaction produced by the "toe warmer" was found to be stable and combine with the extraction protocol the method was found to be comparable, in sensitivity, to laboratory-based methods'.

251 In August 2012, Köser *et al* reported on the practical considerations of the routine use of whole genome sequencing in diagnostic and public health microbiology. The authors noted that it was already becoming cost effective compared to alternative approaches in a number of areas, including 'molecular epidemiology performed for surveillance and outbreak investigation and genotypic antimicrobial susceptibility testing for microbes that are difficult to grow'.

252 An August 2013 working paper submitted by the United States of America to the BWC Meeting of Experts highlighted 'advances in technology have led to an increased speed of diagnostic test development using nucleic acid sequence information and synthetic biology for manufacturing specific antigens or antibodies used in those tests'.

253 In February 2013, researchers at Georgia Tech reported having developed a Computational Genomics Pipeline capable of analysing sequence data from foodbourne pathogens such as *E.coli*, and 'provides clues as to which genes are involved in making people sick. Manually, this process used to take weeks, months or a year. Now it takes us about 24 hours'.

254 In April 2013, Munro *et al* reported the development of a novel cheaper, high-throughput friendly assay to test for certain influenza subtypes. The new assay uses Luminex microarray hybridization. Its efficacy was tested against traditional reverse transcription PCR. The authors demonstrated a sensitivity of over 89% on all subtypes tested. Their assay had a comparable sensitivity when compared to RT-PCR for about half of the sub-types but was about 10-fold higher for the others.

255 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted 'rapid advances in a number of enabling technologies including the '-omics', bioinformatics, systems biology and immunology have assisted the development of new strategies, allowing the identification of new targets and reducing the timescale for vaccine development'.

256 A January 2013 review of computational drug repositioning highlighted progress in 'computational techniques for systematic analysis of transcriptomics (Connectivity Map, CMap), side effects, and genetics (genome-wide association study, GWAS) data to generate new hypotheses for' drug targets.

257 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted:

- 'Research on neuropeptides and bioregulators is providing an increased understanding of how these molecules work, and advances in synthetic biology enable the possibility of large-scale production of toxins, bioregulators, and other biologically active molecules that could be used for hostile purposes '; and
- 'High-Throughput Toxicological Screening… Chemical genomics can either be target-based (often using protein binding assays or reporter gene assays) or cell-, organism-, or tissue- based (phenotype-based screening). Trends in this area include the use of techniques based on robotic systems and the use of large, validated single-compound libraries. This indicates that the science has moved away from the use of combinatorial libraries. In addition, there has been a marked decrease in the scale of traditional assays (from volumes of ~50 to ~4 µL) with faster throughput'. The authors noted 'methodological changes have had implications for the outputs of high-throughput screening. Readouts are providing information on a bigger scale, i.e., an understanding of the effects on the whole organism is being sought, not just on specific molecular targets. A crucial area of research is in predictive modeling methods to improve the extrapolation of data from in vitro to *in vivo* (systems pharmocology)'.

258 A June 2014 review of the implications of the convergence of biology and chemistry highlighted 'drug companies have screened large numbers of metabolically resistant analogues [of peptides], mostly with unnatural (chemical) modifications, some of which may have substantially increased potency and toxicity. However, such modifications may add to the complexity and cost of the end product'.

259 In May 2011, Fleishman *et al* reported having developed 'a general computational method for designing proteins that bind a surface patch of interest on a target macromolecule'. The authors used their method to design 'proteins that bind a conserved surface patch on the stem of the influenza hemagglutinin (HA) from the 1918 H1N1 pandemic virus'. Two of the designed proteins bound to the target with low nanomolar affinity and crystal structures of one bound to the target were 'nearly identical to that in the computational design model'.

260 In June 2014, Procko *et al* reported induces apoptosis in cells infected with the Epstein-Barr virus using a computationally designed inhibitor of a viral bcl-2 protein. Computational design and experimental optimization were used to generate a novel protein called BINDI that binds the gene that the virus uses to block apoptosis with picomolar affinity. The authors note that 'high-specificity-designed proteins that selectively kill target cells may provide an advantage over the toxic compounds used in current generation antibody-drug conjugates'.

261 In March 2012, Tatonetti *et al* reported having developed a data-driven prediction of drug effects and interactions. The authors used an adaptive data-driven approach to correct for unknown or unmeasured variables combined with existing methods to improve analyses of drug effects and drug-drug interactions. The authors demonstrated 'the biological use of these new resources… to identify drug targets, predict drug indications, and discover drug class interactions. We then corroborated 47 ($P < 0.0001$) of the drug class interactions using an independent analysis of electronic medical records'.

262 A July 2012 presentation by Pitt to the BWC Meeting of Experts highlighted a range of advances and technologies in the life sciences, including effective protein engineering leading to designer proteins and protein binding agents, and 'New detection technologies (e.g. peptide aptamers, SOMAmers, etc.)'.

263 A March 2013 review of bioinformatics approaches to identify drug-drug interactions (DDIs) highlighted progress in 'the construction of databases for efficient searching of known DDIs to the prediction of novel DDIs based on data from electronic medical records, adverse event reports, scientific abstracts, and other sources'.

264 In March 2013, Huang *et al* reported having developed a tool for the systematic prediction of pharmacodynamic drug-drug interactions (PD DDIs) through protein-protein-interaction networks. The authors used the tool to predict 9,626 potential PD DDIs at the accuracy of 82%.

265 In December 2013, Guimerà and Sales-Pardo reported having developed a network inference algorithm to predict uncharacterized drug-drug interactions. The algorithm makes use of 'sets of previously reported interactions, and does not require any pharmacological or biochemical information about the drugs, their targets or their mechanisms of action'. The authors assert this approach 'can deal with adverse interactions, synergistic/antagonistic/suppressing interactions, or any other type of drug interaction'. They show that this method 'is able to accurately predict interactions, both in exhaustive pairwise interaction data between small sets of drugs, and in large-scale databases'. The authors demonstrate that the algorithm 'can be used efficiently to discover interactions of new drugs as part of the drug discovery process'.

266 In October 2014, Cheng and Zhao reported the application of machine learning to identify drug-drug interactions (DDIs). The authors demonstrated that 'machine learning-based integration of drug phenotypic, therapeutic, structural, and genomic similarities, we demonstrated that HNAI [heterogeneous network-assisted inference] is promising for uncovering DDIs in drug development and post-marketing surveillance'.

267 In March 2015, Jiang *et al* reported applying Semantic Web-based approach for mining severe drug-drug interaction-induced adverse drug events (ADEs). The authors demonstrated their approach could be used to identify 601 such adverse events involving three frequently prescribed cardiovascular drugs. The authors argue that 'the approach developed could be generalized to detect the signals of putative severe ADEs induced by DDIs in other drug domains and would be useful for supporting translational and pharmacovigilance study of severe ADEs'.

268 A November 2013 review of an automated tool to assist in drug discovery highlighted 'software that read tens of thousands of research papers and then predicted new discoveries about the workings of a protein that's key to cancer could herald a faster approach to developing new drugs'.

269 In February 2014, Wijma *et al* reported computationally designed libraries for rapid enzyme stabilization. The authors constructed 'a library with chemically diverse stabilizing mutations [which] allows the engineering of drastically stabilized and fully functional variants of the mesostable enzyme limonene epoxide hydrolase'. The authors experimentally screened the library and revealed 21 (pairs of) stabilizing mutations. They then combined '10 – 12 of these confirmed mutations resulted in multi-site mutants with an increase in apparent melting temperature from 50 to 85°C, enhanced catalytic activity, preserved regioselectivity and a >250-fold longer half-life'.

270 A September 2014 review of the integration of big data approaches into systems biology and systems pharmacology stressed the benefits of combining data that report how drugs affect the phenotype of human cell lines, how drugs induce changes in gene and protein expression in human cell lines, knowledge about human disease, side effects induced by drugs, and mouse phenotypes. The authors highlighted progress in the application of single-node-type networks, gene-set libraries, or multipartite graphs. They argue 'this approach can lead us to the identification of more relationships between genes, drugs, and phenotypes as well as benchmark computational and experimental methods'.

271 An October 2014 review of the convergence of biology and chemistry highlighted:

- Using directed evolution 'to take existing, non-optimal enzymes and tailor their properties to drive chemical reactions with high selectivity and yield';
- Increasing miniaturization and automation, combined with the use of standardised biological parts – for example 'a "toolbox" of sequences that have specific effects (gene sequence promoters, tags, terminators, etc.) were identified and catalogued to allow for easier sequence development'. The authors noted such an approach 'increases quality control, aids in records keeping and project tracking, and has increased the rate of sequence development significantly. This has also reduced the technical skill level required to perform the synthesis'. The authors also highlighted the potential to combine this approach with directed evolution;
- Progress in developing antibody-drug conjugates for the specific delivery of cytotoxic payloads. The authors noted that such an approach 'combines selectivity with toxicity, by linking antibodies and cytotoxic molecules together'. The authors highlighted a Swiss company which 'has developed both the capacity to produce bulk quantities and the specialist skills and experience in both the chemical and biotech fields necessary to manufacture' these compounds;
- 'Using one-step synthesis, it is possible to generate nanoparticles coated with arrangements of hydrophobic and hydrophilic compounds on a length scale similar to biological materials'. The authors noted that 'these nanoparticles have been shown to have the ability to penetrate cell membranes. Such nanoparticles could either be designed to carry small payloads of highly active drugs (for ex- ample certain peptides), or the nanoparticle itself could act as a drug by interacting with viruses'.

272 A January 2012 review of biomedical applications of synthetic biology highlighted development for:

- Drug discovery, including antibiotics, drugs to reverse antibiotic resistance, anti-diabetes drugs, and immunosuppressants; and
- Cancer therapies, including bacterial synthetic devices, viral synthetic devices, and a transformation sensor for cancer therapy.

273 A 2012 review of technologies for studying cells highlighted "describe bioengineering approaches for controlling and measuring cell-environmental interactions in vitro, including strategies for high-throughput analysis". The authors reported progress in the "reduction of complex multicomponent cellular microenvironments into distinct individual signals". The authors conclude that these tools are enabling a wide range of applications, including "in fundamental biological studies, in vitro modeling of *in vivo* processes, and cell-based therapies.

274 In August 2012, Prindle *et al* reported having successfully transitioned gene circuits designed for use in E. coli into *Salmonella typhimurium* which they argue is "a therapeutically relevant microbe with attenuated strains that have exhibited safety in several human clinical trials". Such a cassis is intended to assist applying synthetic biology approaches in a public health setting.

275 A November 2013 review of applying synthetic biology approaches to chemical engineering identified a range of chemicals being produced using bio-based systems, including: 1, 4 – butanediol, a high-value chemical used as a solvent and in the manufacture of plastics and fibers; and n-butanol, commonly used in surface coatings like paints. The authors also detailed the Gen9 BioFab platfor, developed for the large-scale production of genetic material.

276 In January 2011, Fisher *et al* reported testing a library of de novo designed proteins for biological function in *E.coli*. The authors reported having 'probed the capacity of proteins from this library to function *in vivo* by testing their abilities to rescue 27 different knockout strains of *Escherichia coli*, each deleted for a conditionally essential gene'. They identified proteins capable of rescuing four of the knockout strain.

277 In December 2011, Miller *et al* reported high-resolution dose–response screening using droplet-based microfluidics. The authors presented an approach that increased the number of data points from 7-10 to approximately 10,000 per compound enabling highly precise and reproducible assessments of drug efficiency and revealing complex dose–response relationships. The authors used their approach to identify a number of novel inhibitors for a protein associated with diabetes, obesity, and cancer.

278 Online laboratories are beginning to offer remote research platforms. For example, Emerald Cloud Lab offers 42 functions at present from flow cytometry, through protein extraction to transfection. Alternatively, Transcriptic offers a whole of life cycle process providing experimental design, automated research, and results analysis.

279 A March 2011 review highlighted 'a chip-based method that creates uniformly sized vesicles in assembly-line fashion. Sized between 20 and 70 micrometers in diameter, the vesicles are large enough to be loaded with DNA and the biochemical machinery to act as synthetic cells. The synthetic packaging will help researchers study the proteins in cell membranes, which play important roles as gatekeepers of the cell. Many drugs, for example, act on these membrane proteins or otherwise use them to get inside cells in order to do their job'.

280 In September 2012, Alsford *et al* reported using high-throughput processes to decode the efficacy and resistance of drug used to treat African trypanosomiasis. The authors reported using 'genome-scale RNA interference target sequencing (RIT-seq) screens in Trypanosoma brucei, revealing the transporters, organelles, enzymes and metabolic pathways that function to facilitate antitrypanosomal drug action'. They noted 'advances in our understanding of mechanisms of antitrypanosomal drug efficacy and resistance will aid the rational design of new therapies and help to combat drug resistance, and provide unprecedented molecular insight into the mode of action of antitrypanosomal drug'.

281 In December 2013, Molinelli *et al* reported experimental-computational technology for inferring network models that predict the response of cells to perturbations. The model focuses on the use of targeted drugs, singly or in combination, to affect cancer cell lines. The affect 'is quantified in terms of relative changes in the measured levels of proteins, phospho-proteins and cellular phenotypes such as viability'. Computational network models explore the 'large solution space of all possible network models' using a probabilistic algorithm. The authors used their model to identify a previously unidentified perturbation that would result in the inhibition of a key drug target in a melanoma cell line, which was subsequently confirmed experimentally.

282 In October 2014, Platt *et al* reported generated mouse line with endogenous expression of the CAS9 proteins enabling CRISPR based genome editing using only guide RNAs. The authors demonstrated an ability to edit targets in the brain, vasculature, immune cells, and lung. They used the mouse model to investigate competition between gain- and loss-of-function mutations in lung cancer.

283 A September 2014 review of mRNA-based therapeutics describes efforts to utilise In vitro transcribed (IVT) mRNA to deliver genetic information. The authors note that 'such synthetic mRNA can be engineered to transiently express proteins by structurally resembling natural mRNA'. Those proteins can be used to combat infectious disease and several clinical applications are under development. The authors also describe efforts for the '*in vivo* delivery of IVT mRNA to replace or supplement proteins, IVT mRNA-based generation of pluripotent stem cells and genome engineering using IVT mRNA-encoded designer nucleases'.

284 In August 2011, Saeidi *et al* reported having engineering microbes to sense and eradicate Pseudomonas aeruginosa. The authors reported 'the development of a synthetic genetic system, which comprises quorum sensing, killing, and lysing devices, that enables *Escherichia coli* to sense and kill a pathogenic... strain through the production and release of pyocin. They demonstrated that the introduction of their engineered organism led to a 99% decrease in the pathogen and that it 'inhibited the formation of P. aeruginosa biofilm by close to 90%, leading to much sparser and thinner biofilm matrices'.

285 A January 2012 review of biomedical applications of synthetic biology highlighted development for:

- Novel treatments for infection, including 'breaking bacterial resistance by designer phages', and engineered probiotic bacteria to decrease pathogen virulence; and
- Other tools for biomedicine, including RNA controllers of cell proliferation, Optogenetic devices in blood glucose homeostasis, prosthetic networks, and an artificial insemination device.

286 An October 2014 review of the convergence of biology and chemistry highlighted:

- Research to use CRISPR/CAS9 gene editing technologies to treat disease. The authors noted 'experiments on monkeys inactivating the genes for two human diseases, which poses the question of potential applications in humans';
- 'Using one-step synthesis, it is possible to generate nanoparticles coated with arrangements of hydrophobic and hydrophilic compounds on a length scale similar to biological materials'. The authors noted that 'these nanoparticles have been shown to have the ability to penetrate cell membranes. Such nanoparticles could either be designed to carry small payloads of highly active drugs (for ex- ample certain peptides), or the nanoparticle itself could act as a drug by interacting with viruses';
- 'Advanced computational methods and directed evolution are used to develop enzymes tailored to address a specific industrial need... Given recent advances, it is now possible to take advantage of the tools of nature and those of molecular modelling to take existing, non-optimal enzymes and tailor their properties to drive chemical reactions with high selectivity and yield'. The authors noted that 'this work may be useful is in treatment or deactivation of harmful materials either *in vivo* or as part of decontamination'.

287 In July 2015, Schumann *et al* reported a significant step towards the clinical application of CRISPR/CAS9 genome editing. The authors reported having developed 'a programmable tool to replace specific nucleotide sequences in the genome of mature immune cells'. They noted such an approach 'holds great promise for cancer immunotherapies and cell-based therapies for HIV, primary immune deficiencies, and autoimmune diseases'.

288 In June 2015, Ramanan *et al* reported a novel therapeutic approach using genome editing tools to directly cleave viral DNA, thereby promoting viral clearance. The authors used a CRISPR/Cas9 system to specifically target Chronic hepatitis B virus, demonstrating "cleavage of cccDNA by Cas9 and a dramatic reduction in both cccDNA and other parameters of viral gene expression and replication".

289 In August 2014, Thi *et al* reported having used a lipid-encapsulated siRNA to treat non-human primates infected with Marburg virus. The authors demonstrated that post infection treatment of the drug from 30 minutes to three days after infection resulted in 100% survival of the monkeys, whilst all those untreated, or treated with a control died between 7 and 9 days after infection.

290 A February 2015 review of the *in vivo* challenges of RNAi therapeutics highlighted issues around the specific, efficient and targeted delivery of siRNAs. The authors noted 'anatomical barriers, drug stability and availability, immunoreactivity and existence of various delivery routes, different genetic backgrounds are major clinical challenges'.

291 An April 2012 review of therapeutic applications of siRNA highlighted progress in addressing such challenges as 'stability, potency, off-target effects, and efficient delivery of synthetic siRNAs'. The authors noted a number of therapeutics currently in development, including a number in Phase I and Phase II clinical trials.

292 An August 2011 review of monoclonal antibody therapies against Anthrax highlighted progress in developing antibodies 'to each of the virulence components: protective antigen (PA), lethal factor (LF) and oedema factor (EF), and the capsule of B. anthracis'.

293 In September 2014, Kammanadiminti *et al* reported that a combined therapy consisting of antibiotics and anthrax immune globulin intravenous is potentially more effective than antibiotics alone in rabbit model of inhalational anthrax. The authors demonstrated that whilst the combination therapy had little impact when treatment occurred 60 hours after exposure, more animals survived when treatment was delayed.

294 In March 2015, the US Federal Drug Administration 'approved Anthrasil, Anthrax Immune Globulin Intravenous (Human), to treat patients with inhalational anthrax in combination with appropriate antibacterial drugs'.

295 In August 2013, Pettitt *et al* reported the successful use of a monoclonal antibody cocktail to treat non-human primates exposed to Ebola virus. The treatment led to a 43% survival rate, rather than a standard 0% for the viral challenge.

296 In March 2014, Krammer *et al* reported the results of an assessment of influenza virus hemagglutinin stalk-based immunity in ferrets. The authors demonstrated the efficacy of a pan-H1-reactive monoclonal antibody against a pandemic H1N1 challenge virus in the ferret model of influenza disease. The authors also reported 'a universal influenza virus vaccine strategy based on chimeric hemagglutinin constructs that focuses the immune response on the conserved stalk domain of the hemagglutinin'. The demonstrated 'both strategies showed efficacy in reducing viral loads after an influenza virus challenge in the ferret model'.

297 In December 2012, the US Federal Drug Administration approved the monoclonal antibody-based therapy raxibacumab for use to treat inhalational anthrax. It was the first use of the animal efficiency rule to approve such a drug.

298 A March 2015 review of Anthrax therapeutics development and pipeline highlights monotherapy and combination therapies, as well as new entrants to the market and the emerging players. The authors identified 33 companies currently developing anthrax therapeutics. Collectively they are working on 39 different products.

299 In December 2011, Koehler *et al* reported having developed a new approach to identify modified Type I interferons that demonstrate elevated, broad-spectrum antiviral activity. Type I interferons are 'released from infected cells bind to a receptor... on neighbouring cells, triggering signalling cascades that limit further infection'. The authors used 'gene crossbreeding method to generate hybrid, type I human IFNs with enhanced antiviral activity against four dissimilar, highly pathogenic viruses. Approximately 1400 novel IFN genes were expressed in plants, and the resultant IFN proteins were screened for antiviral activity'.

300 In September 2011, Côté *et al* reported having identified a new drug target to combat Ebola virus. The authors demonstrated that the target, Niemann–Pick C1, was essential for Ebola virus entry into cells.

301 In April 2014, Nishizawa *et al* reported having identified 'a new type of antiprion compound, Gly-9,... found to inhibit abnormal prion protein formation in prion-infected neuroblastoma cells' following more than 1 day of treatment. The authors demonstrated 'it reduced the intracellular prion protein level and significantly modified mRNA expression levels of genes of two types... after more than 2 days of treatment. They noted that whilst '*in vivo* efficacy of Gly-9 was limited, the findings for Gly-9 provide insights into the regulation of abnormal prion protein in cells and suggest new targets for antiprion compounds'.

302 In April 2013, Perry *et al* reported having identified a novel drug candidate for the treatment of Dengue virus. The authors demonstrated the compound 'reduced mortality, as well as viremia and viral RNA in key tissues, and cytokine storm. In addition... treatment can be delayed'.

303 In May 2013, Bian *et al* reported having established invasive, inheritable infections of a strain of Wolbachia bacteria in the important malaria mosquito vector, Anopheles stephensi. The particular strain of Wolbachia bacteria 'conferred resistance in the mosquito to the human malaria parasite *Plasmodium falciparum*'.

304 An August 2014 presentation to the BWC Meeting of Experts highlighted the potential to use CRISPR/CAS9 gene drives to influence the inheritance of genes in sexually reproducing animals and plants with short reproduction cycles. The author noted implications for: eradication of disease, such as removing the ability of mosquitoes to act as vectors for malaria or dengue fever; suppression of invasive species; reduction of herbicide resistance; and the development of immunization drives and reversal drives.

305 An August 2013 review of efforts to develop a tularaemia vaccine highlighted progress resulting from 'a substantial increase in knowledge of the pathogenic mechanisms of the organism and the induced immune responses'.

306 A June 2014 review of vaccine design technologies highlighted both successes and failures and an increasing focus on iterative approaches. The authors review 'novel antigens, adjuvants and vectors in the preclinical stage with computational analyses of clinical data to accelerate vaccine design'. They conclude that 'reverse and structural vaccinology have revealed novel antigen candidates and molecular immunology has led to the formulation of promising adjuvants. Gene expression profiles and immune parameters in patients, vaccinees and healthy controls have formed the basis for biosignatures that will provide guidelines for future vaccine design'.

307 A July 2014 review of vaccine design highlighted 'innovative technologies currently used in vaccine research and development including adjuvants, vectors, nucleic acid vaccines, and structure- based antigen design'.

308 In December 2013, Lua *et al* reviewed the application of engineering principles to the rational design of virus-like particles (VLPs). VLPs attempt to 'harness the optimally tuned immunostimulatory properties of natural viruses while omitting the infectious trait'. They have been proven to be safe and highly effective in humans. The authors note significant progress in being able to 'specifically engineer a desirable immune response through modular VLP design, and those that seek to improve bioprocess efficiency through inhibition of intracellular assembly to allow optimal use of existing purification technologies prior to cell-free VLP assembly'. They note that a greater understanding of VLP assembly is required but predict that economic drivers and cross-discipline research will made significant inroad over the coming decade.

309 A July 2014 review of engineering strategies to construct immunogenic live vaccines highlighted progress in 'the adaptation of attenuated strains to create multivalent vaccine platforms for immunization against multiple unrelated pathogens. These carrier vaccines are engineered to deliver sufficient levels of protective antigens to appropriate lymphoid inductive sites to elicit both carrier-specific and foreign antigen-specific immunity'. The authors stressed 'that the ultimate success of an engineered vaccine rests on achieving the proper balance between attenuation and immunogenicity. Achieving this balance will avoid over-activation of inflammatory responses, which results in unacceptable reactogenicity, but will retain sufficient metabolic fitness to enable the live vaccine to reach deep tissue inductive sites and trigger protective immunity'.

310 In November 2014, Khurana *et al* reported 'that N-terminus β sheet domain-swap can be used to produce stable functional oligomeric forms of better recombinant HA1 vaccines in simple, inexpensive bacterial system for rapid response to emerging pandemic threat for the global population'. The authors demonstrated that influenza vaccine challenge studies revealed better protection of ferrets from lethality, weight loss, and reduced viral loads and 'antibody affinity maturation far superior to the inactivated H7N7 subunit vaccine'.

311 In a March 2012 review of progress in developing conjugate vaccines, Dro noted that significant progress has been made in the application of bio-based design and production. He notes that recombinant DNA technologies allows for 'the controlled design and production of glycoproteins with customised polysaccaride structure, which will target bacterial pathogens that cannot be address with existing chemical processes'. The author records three potential advantages of a bio-based approach: the versatility of expressing specific polysaccarides and protein antigens; the ability to express epitopes in an almost native form; and benefits for the consistency of products. Dro also notes that using a novel design and production platform does pose regulatory challenges in demonstrating efficacy and safety.

312 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted a number of developments in vaccine design, including:

- 'Design of self-assembling influenza nanoparticle vaccines that elicit broader and more potent immunity than traditional influenza vaccines';
- 'In research on HIV vaccine design, which utilised computational prediction and directed evolution to engineer an immunogen that would elicit production of broadly neutralising antibodies. A self-assembling virus-like nanoparticle was designed and coated with multiple copies of the optimised immunogen to achieve better stimulation of the antibody response';
- 'Demonstration of the potential of DNA nanostructures to serve as general platforms for the rational design and construction of a variety of vaccines';
- 'Development of a synthetic approach that rapidly generated influenza vaccine viruses from sequence data';
- 'Creation of a synthetic vaccine for foot and mouth disease';
- Completion of Phase I clinical testing of a monoclonal antibody to 'a surface polysaccharide common to a diverse range of microbial pathogens, including bacterial, fungal and protozoan examples, which could provide a promising target for the development of a broad spectrum vaccine'.

313 A January 2012 review of biomedical applications of synthetic biology highlighted development for:

- Improved vaccines, including for chikungunya virus, *Staphylococcus aureus* and antigen-producing immunostimulatory liposomes as genetically programmable synthetic vaccines; and
- Vector control, including for mosquitoes.

314 In October 2014, Shen *et al* reported using synthetic biology techniques to develop a methodology for synthetic attenuated virus engineering. The authors demonstrated that changes to the preferential usage of certain underrespresented codons and codon pairs to encode amino acids and adjacent amino acid pairs can attenuate viruses in both cell culture and mouse models, in a tunable fashion. They used this approach to produce attenuated polioviruses and influenza viruses, HIV retroviruses and the bacterium, Streptococcus pneumonia.

315 In April 2014, Warren *et al* reported having used a novel broad-spectrum nucleoside analogue to protect against filovirus infections. The compound inhibits infection of distinct filoviruses in human cells by inhibits viral RNA polymerase function. The authors demonstrated:

- 'Post-exposure intramuscular administration... protects against Ebola virus and Marburg virus disease in rodent models...';
- Completely protects cynomolgus macaques from Marburg virus infection when administered as late as 48 hours after infection...';
- Exhibits broad-spectrum antiviral activity against numerous viruses, including *bunyaviruses*, *arenaviruses*, *paramyxoviruses*, *coronaviruses* and *flaviviruses*'.

316 A February 2015 review of RNA-based viral vectors highlighted progress in exploiting 'the basic replication and expression strategies of RNA viruses to produce vaccine antigens that have been engineered into their genomes'. The authors note that there has been 'significant preclinical testing of many RNA virus vectors against a wide range of pathogens as well as cancer targets. Multiple RNA virus vectors have advanced through preclinical testing to human clinical evaluation'.

317 In March 2013, Porta *et al* reported having expressed recombinant empty viral capsid, rationally redesigned to negate the need for a cold-chain, for use as a vaccine against Foot-and-Mouth Disease. The authors demonstrated that the capsids did confer immunity from the disease when challenged in cattle as 34 weeks. They asserted that this 'approach to vaccine antigen production has several potential advantages over current technologies by reducing production costs, eliminating the risk of infectivity and enhancing the temperature stability of the product'. They also noted the potential to use the approach to produce vaccines for other pathogenic picornaviruses in humans and animals.

318 In November 2014, Rincón *et al* reported identifying electrostatic repulsion inside the capsid of Foot-and-Mouth Disease viruses as being responsible for its thermolability. They rationally redesigned the capsid to increase thermostability. This was intended to facilitate the developments of more efficient vaccines but could potentially also be used to produce a more environmentally resistant virus.

319 In July 2013, Kanekiyo *et al* reported having designed and built self-assembling influenza nanoparticle vaccines against influenza. 'The viral haemagglutinin was genetically fused to ferritin, a protein that naturally forms nanoparticles composed of 24 identical polypeptides. Haemagglutinin was inserted at the interface of adjacent subunits so that it spontaneously assembled and generated eight trimeric viral spikes on its surface'. The authors demonstrated that these particles 'elicited haemagglutination inhibition antibody titres more than tenfold higher than those from the licensed inactivated vaccine. Furthermore, it elicited neutralizing antibodies to two highly conserved vulnerable haemagglutinin structures that are targets of universal vaccines: the stem and the receptor-binding site on the head'.

320 In September 2013, Smith *et al* reported developing a virus like particle for use as a vaccine against influenza H7N9 and H7N3. The authors demonstrated 100% survival of animals in a vaccine challenge study compared to 0% with alternate influenza vaccines and placebos. They note 'the data demonstrate that recombinant H7N9 vaccine can be rapidly developed that was immunogenic and efficacious supporting testing in man as a pandemic influenza H7N9 vaccine candidate'.

321 In July 2014, Zhang *et al* reported the licensing of a Virus-Like Particle as a vaccine against Hepatitis E and related diseases. They reported a 50-fold increase in particle production in scale up from bench to manufacturing.

322 In June 2014, Ruiz *et al* reported and compared a number of different expression systems in Baculovirus Vectors for creating empty Foot-and-Mouth Disease virus capsid for use as vaccines.

323 In May 2015, Deng *et al* reported a significant step towards a universal vaccine against Influenza A viruses. The authors reported fusing a conserved influenza surface protein to a major coat protein of filamentous bacteriophage f88. They demonstrated that the recombinant virus was replication competent and displayed the desired protein. Mice immunized with the recombinant virus in the presence of an adjuvant were protected against challenge with human and avian influenza A viruses.

324 A December 2013 review of promising approaches for short term improvements in influenza vaccines highlighted:

- 'Technological advances such as mammalian cell culture production and synthetic vaccine seeds provide a means to increase the speed and accuracy of targeting new influenza strains with mass-produced vaccines by dispensing with the need for egg isolation, adaptation, and reassortment of vaccine viruses.
- New influenza potency assays that no longer require the time-consuming step of generating sheep antisera could further speed vaccine release.
- Adjuvants that increase the breadth of the elicited immune response and allow dose sparing provide an additional means to increase the number of available vaccine doses'.

325 A July 2014 review of the use of adjuvants to enhance the efficacy of inactivated influenza vaccines noted that 'Oil-in-water adjuvants like MF59 and AS03 have been licensed and widely used, and shown efficacious in preventing influenza infection in the last pandemic. MF59-adjuvanted inactivated vaccine was more efficacious than non-adjuvanted vaccine in preventing influenza infection in young children and in reducing hospitalization due to the influenza infection in the elderly.' These are the groups in which such vaccines have traditionally had suboptimal efficacy.

326 In October 2014, Mulligan *et al* reported the results of a Phase I clinical trial that found 'point-of-use mixing of H5N1 antigen and MF59 adjuvant achieved target antibody titers in a high percentage of subjects and was safe'. The authors demonstrated that the target antibody levels were achieved in 80% of subjects receiving both the vaccine and the adjuvant, compared to only 14% of subjects receiving only the vaccine. Neutralizing titers were 2- to 3-fold higher with the adjuvant than without and the pairing produced cross-reactive antibody responses against 4 H5N1 viruses.

327 A February 2015 review of key milestones in the discovery and development of self-amplifying mRNA vaccines and their potential to be used as a rapid response platform concluded that 'the prospects for non-viral delivery of self-amplifying mRNA vaccines are very promising. Like other types of nucleic acid vaccines, these vaccines have the potential to draw on the positive attributes of live-attenuated vaccines while obviating many potential safety limitations'. The authors highlight preclinical proof of concept for vaccine candidates against influenza, respiratory syncytial virus, rabies, Ebola, cytomegalovirus, human immunodeficiency virus and malaria.

328 In May 2013, Blaney *et al* reported data on antibody quality and protection from lethal Ebola virus challenge in nonhuman primates immunized with rabies virus based bivalent vaccine.

329 A March 2012 review of developments in vaccine production highlighted:

- The use of insect cell line to grow virus like particles – proteins that mimic viral structure without any genetic material;
- The use of disposable equipment, both disposable bags inside stainless steel incubators to grow the vaccine and disposable parts in the purification system;
- The use of human cell lines to produce vaccine, allowing continuous vaccine production 'without having to restart cell cultures from scratch every 100 generations';
- Directed evolution to remove reliance on animal serum, reducing cost, stabilising ingredient chains, and assisting in meeting purification standards;
- The use of suspended cultures which reduces the physical space required for vaccine production and significantly reducing cost by enabling batch processing of verification steps, and dramatically increasing surge production capacity.

330 In August 2013, Mignaqui *et al* reported using transient gene expression in serum-free suspension-growing mammalian cells for the production empty foot-and-mouth disease viral capsids as vaccine candidates. The authors report this approach 'is an easy to perform, scalable and cost-effective technology for the production of a recombinant subunit vaccine against FMDV'. The same month, Gullberg *et al* reported a second way to produce self-assembly viral capsids using a vaccinia-virus-based transient expression system. The authors characterised the physical structure of the capsids and demonstrated an immunological response.

331 In January 2013, the US Federal Drug Administration licensed the first recombinant, highly purified, egg-free influenza vaccine. It was also the first influenza vaccine grown using insect cells to get regulatory approval. The process did not involve growing any influenza virus, offering biosafety benefits.

332 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted:

- 'The production of toxic chemicals, including toxins, through biological synthesis (an aspect of the convergence of chemistry and biology); encapsulation and delivery through nanotechnology; and flow microreactors, which enable types of chemical reactions under conditions that were not previously technically feasible';
- 'Microreactors have now entered the chemical production toolkit for certain kinds of operations. They alter the signature of chemical production infrastructure to some extent, although they are only one piece of a sequence of infrastructure'. The authors noted the 'major advantages of microreactor technology include the safe production of hazardous, corrosive chemicals, as well as certain classes of biologically active chemicals, which could not be otherwise done under batch conditions'. They also highlighted they 'could shorten the time from the discovery of a new class of toxic chemicals to production of selected agents. There is also the possibility that other toxic chemicals (e.g., pharmaceuticals and peptides) could be produced in microreactors for prohibited purposes'.

333 A December 2014 review by Lin *et al* of the use of baculovirus/ insect cell expression systems as production platforms for recombinant proteins had been extensively used for the production of various vaccine candidates, and several commercially-available human and veterinary vaccine products. The authors also note that as baculovirus is capable of entering a broad range of mammalian cells, it is also a promising gene delivery vehicle for *in vivo* antigen expression and display.

334 A January 2015 review of the security implications of synthetic biology highlighted the impact of such developments on the production of narcotics. The authors noted 'the potential for synthetic biology to enable, and be enabled by, a distributed production capacity could also impact upon the existing supply chains for illegal narcotics. For example, an engineered yeast capable of producing such narcotics would offer a very different footprint to agricultural or chemical production approaches. It may even lend itself to relocating production closer to the end user, diminishing the need for illicit trafficking of bulk material, thereby complicating interdiction'.

335 A May 2015 commentary on the recent publication of missing steps in the opinoid metabolic pathway noted 'yeast-based production of opiates could provide an alternative system for current criminal networks, particularly in North America and Europe, where the drugs are in high demand. Because yeast is easy to conceal, grow and transport, criminal syndicates and law-enforcement agencies would have difficulty controlling the distribution of an opiate-producing yeast strain. All told, decentralized and localized production would almost certainly reduce the cost and increase the availability of illegal opiates — substantially worsening a worldwide problem'.

336 In May 2013, Dormitzer *et al* reported having used sequencing and synthesis to deliver a vaccine candidate to the producer 18 days faster than a second group using traditional recombinant DNA techniques. The authors demonstrated increasing the yield of the essential vaccine antigen. They noted that 'generation of synthetic vaccine seeds, together with more efficient vaccine release assays, would accelerate responses to influenza pandemics through a system of instantaneous electronic data exchange followed by real-time, geographically dispersed vaccine production'.

337 In July 2014, Dormitzer described a synthetic approach to influenza vaccine virus generation in which 'viral sequence data from many sources are posted on the Internet, are downloaded by vaccine manufacturers, and are used to rescue multiple, attenuated vaccine viruses directly on high yielding backbones'. The author notes that 'elements of this system were deployed in the response to the 2013 H7N9 influenza outbreak in China. The result was the production, clinical testing, and stockpiling of an H7N9 vaccine before the second wave of the outbreak struck at the end of 2013'.

338 An August 2013 presentation by Sanofi to the BWC Meeting of Experts highlighted drug production efforts to scale up and produce a semi-synthetic artemisinin. The authors noted the steps taken to optimise the strain producing the precursor for industrial production, optimise fermentation, and optimization of the down stream process. Industrial production was then outsourced to a multipurpose facility in Bulgaria. The precursor produced by a yeast was then transferred to another facility and traditional industrial chemistry was used for the final step of the synthesis. The author noted that the industrialisation of this product from a functional lab scale was around 4 years.

339 A March 2012 review of developments in vaccine production highlighted the use of disposable equipment, both disposable bags inside stainless steel incubators to grow the vaccine and disposable parts in the purification system.

340 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted a number of developments in vaccine production, including:

- 'Design of self-assembling influenza nanoparticle vaccines that elicit broader and more potent immunity than traditional influenza vaccines' using synthetic nanoparticles eliminating 'the need to grow potentially dangerous viruses in eggs or cell culture, a comparatively costly and time-consuming step of commercial vaccine production';
- 'Single-use or disposable bioreactor systems have also progressed; these are easily installed, reduce costs, streamline validation, increase product consistency and reduce overall turnaround times. Simple rocker bags are suited to cell-culture for virus vaccine production as an alternative to traditional methods of growth in embryonated eggs. Single-use or disposable components also feature increasingly in downstream processing equipment'; and
- 'The widening diversity of vaccine production methods now include: cell cultures and cell suspension bioreactors; recombinant DNA; metabolic engineering and synthetic biology; chemical peptide synthesis; and transgenic animals and plants'.

341 An April 2014 market report on single use bioreactors highlighted:

- 'Single-use bioreactors are already being deployed for high-density cell culture applications (monoclonal antibodies, recombinant proteins, vaccines etc.); newer application areas being researched include stem cells and personalised medicine.
- 17 manufacturers offer around 80-90 models of single-use bioreactors with working volumes ranging from as low as few millilitres to up to 2000L.
- Manufacturing collaborations and acquisitions are rapidly changing the landscape; amongst the 50 odd partnerships we looked at, around 25% were manufacturing collaborations and an additional 25% were mergers / acquisitions.
- Quite recently, many start-ups have sprung up; examples include CerCell, PBS Biotech, Cellexus Limited; these start-ups have launched single-use bioreactors with innovative features and are likely to play an increasingly important role in the future.

- The overall industry could grow close to USD 1 billion in a few years' time; however, this will be driven to a certain extent by the overall growth of the biopharmaceuticals' market'.

342 An October 2014 review of the impacts of convergence of chemistry and biology (Spiez Convergence Report) highlighted that:

- 'Using tools that are already available, including direct insertion of gene sequences and directed evolution, organisms and molecules can be guided to meet a specific purpose within a reasonable timeframe and with a degree of reliability that was not previously possible. A number of examples were presented and discussed for industrial-scale production of complex molecules with application in medicine and elsewhere';
- 'Genomics, transcriptomics, proteomics and metabolomics together provide a set of tools in synthetic biology that can be used to design organisms with desired properties. These tools have been applied in three different industrial projects: The production of lysine – a feed additive – using *Corynebacterium glutamicum*, production of xanthan – a thickener for use in food and personal care products – using *Xanthomonas campestris pv. campestris*, and production of acarbose – a medication used to treat type II diabetes – by *Actinoplanes sp.* For each of these projects it was critical to obtain information about the cellular machinery, from genome to metabolome, in order to rationally approach modifying the organism';
- Progress in industrial biology is helping 'to develop effective, scalable and robust processes that convert a renewable feedstock such as sugar, using a microorganism such as genetically engineered yeast, to a desired chemical compound at industrial scale'. The authors noted an example where 'To reduce time and costs, the company automated and miniaturised as many repetitive R&D processes as possible… Though the infrastructure for automation was expensive to develop (~$200 M), the cost per sequence to develop has dropped exponentially with the implementation of this process, and the rate at which new sequences are developed has increased by magnitudes;
- 'The response of the organisms to the environment of a fermenter is not predictable, and any new organism must be carefully tested to prevent failures at the pilot plant scale. The timeline for development of a new product has been substantially reduced… However, the cost and time required to produce a new viable, scalable method are still high'.

343 A January 2012 review of single-use equipment in industry highlighted:

- Single-use equipment 'began with straightforward applications like media and buffer containers, tubing, and filters. It has taken a while to address the more critical process steps, but even such complex operations as tangential flow filtration are now available in disposable format';
- The use of single-use equipment is growing – one industry study 'estimates that more than 40% of integrated single-use systems are now used in GMP manufacturing'. The authors noted 'several large biotech companies employ fully disposable processing, including chromatography, for smaller, mid-titre, clinical trial manufacturing processes';
- Size is not an issue – 'working volumes for integrated single-use bioprocess containers, systems, and support equipment have steadily risen from sub-1,000-L levels (50 – 500 L) to the 2,000 L range, with prototypes exceeding 5,000 L for some applications';
- Scaling is becoming much less of an issue, for two reasons: (a) 'end-users feel comfortable with "scaling out" through the use of multiple 500 L or 2,000 L systems rather than scaling up to 5,000 L and higher'; and (b) 'targeted therapeutics and higher titres are causing the slow extinction of mega-processes. Those that remain increasingly turn to multiple 1,000 L or 2,000 L disposable bioreactors to mitigate risks associated with batch loss';
- Recent progress in improving standardization, including 'standardized, off-the-shelf assemblies for cell culture harvest and tangential flow filtration, disposable, aseptic, genderless, universal connectors, and non-proprietary films and materials of construction for its bags, connectors, and tubing';
- Recent progress in addressing mixing in single-use equipment – maintaining sterility, avoiding sheer stress and rubbing on the container pose distinct challenges in disposable systems. The authors noted that at least one company has adopted air-based systems for stirring and agitating cells, which 'offers scalability from three litres for lab work, and 80 litres for preclinical and clinical batches. In targeting full-scale GMP production, the company plans to produce a 500 L system later this year, and a 2,500 L bioreactor in 2012'.

344 A November 2011 review of virus safety of biopharmaceuticals quoted industry professionals and regulators as recognising that "that there is no all-encompassing virus detection method and no 'one-(virus cultivation) medium-for-all' to be able to detect all viral contaminants". The authors noted that the risk profile of viral contamination depends upon a variety of factors including: "source of the biological, raw materials used, production systems, purification reagents and excipients". They highlighted three approaches to mitigate this risk: "(1) prevention of access of virus by screening of starting materials (cell banks, tissues, or biological fluids) and raw materials/ supplements used in production processes (culture media, serum supplements, transferrin, etc); (2) incorporation of robust virus clearance steps, into the manufacturing process; and (3) monitoring production using a relevant screening assay. The authors concluded that "zero risk is a myth, but virus safety can be enhanced by incorporation of multiple overlapping virus containment and clearance strategies".

345 An April 2013 review of technologies for improving downstream productivity of bioproduction highlighted Multimodal chromatography as 'a powerful tool for difficult separation challenges'. The authors noted 'a trend over the past five years… to apply multimodal media to the difficult polishing challenges downstream'. They highlighted such technologies as an 'established workhorse' in the purification of antibody fragments. The authors noted that ion-exchange chromatography was an established purification platform for monoclonal antibodies but highlighted advances in resin design that further optimises the system. The authors highlighted developments in cation-exchange resins, such as Eshmuno CPX, and high capacity, salt tolerant resins, such as Life Technologies' POROS system. The authors noted that 'many blockbusters already include HPLC [high performance liquid chromatography] in their purification process'. The authors highlight advances in more user-friendly tools, software and services to adapt the process to specific product need. The authors noted the importance of optimizing virus filters and highlighted the potential to strengthen filtering earlier in the production process.

346 A November 2014 review of UV-C Irradiation as a viral inactivation method for biopharmaceuticals included an evaluation of its effectiveness in inactivating viral pathogens from seven different families. Different viruses proved to have different levels of susceptibility to UV-C inactivation. The author demonstrated that variation in sensitivity was not connected to "virus size; presence or lack of a lipid envelope; or genome type, size, or strandedness". The author concluded that this was " a highly versatile viral inactivation step that can be included in a purification process in a number of ways. Since it is a flow-through operation, adding it to a process is relatively simple".

347 In October 2012, Wigginton *et al* reported the impact of disinfectant on virus function and structural integrity as a viral inactivation mechanism. The authors undertook a quantitative analysis of the damage to a model virus (bacteriophage MS2) from five common virucidal approaches, incldluing heat, UV, hypochlorous acid, singlet oxygen, and chlorine dioxide. The authors demonstrated that "each treatment targets one or more virus functions to achieve inactivation".

348 A June 2014 review of the implications of the convergence of biology and chemistry highlighted:

- 'Biocatalysts (enzymes), usually immobilised, are being increasingly applied in the industrial production of bulk chemicals and pharmaceuticals. To overcome the limitations of naturally occurring enzymes, directed evolution has become an important tool for improving activity, selectivity, solvent tolerance and general robustness';
- Reprogrammed microorganisms have been created 'to produce biofuels by manipulating metabolic and biosynthetic pathways, and the production of drugs that are difficult to synthesise or which otherwise would need to be extracted from scarce raw materials. Recent examples include the production of anthranilic acids such as the anti-allergic tranilast in modified yeast strains, 24 and the production of terpenoid compounds which form the basis of perfumes and many drugs, including novel antimicrobial drugs';
- 'A survey of synthetic biology products published in 2012 provides additional insight into applications under development.30 The survey identified 68 products across seven sectors (including biofuels, chemicals, energy, food, materials, and medicine) being developed by companies in 10 countries';
- 'Substantial advances in production methods for peptides have been made in the last two decades. Although they could be produced in genetically modified organisms, the pharmaceutical industry currently regards chemical synthesis as the most cost-effective method for producing many small peptides'.

349 A January 2012 review of biomedical applications of synthetic biology highlighted development for:

- Drug production and delivery, including he biosynthesis of new secondary metabolites with novel therapeutic activities (such as complex polyketides, halogenated alkaloids, and the precursors of the anti-malaria drug artemisinin), biohybrid materials for drug delivery; and
- Other tools for biomedicine, including RNA controllers of cell proliferation, Optogenetic devices in blood glucose homeostasis, prosthetic networks, and an artificial insemination device.

350 An August 2014 presentation by Sweden to the BWC Meeting of Experts highlighted the challenges of developing measures to 'surveil development, manufacturing and marketing of a dual use agent with a substantial commercial value'. The authors noted both existing issues with the commercial production of botulinum toxin and those emerging from the adoption of synthetic biology techniques.

351 A February 2015 review by Yang *et al* of engineered biomaterials for development of nucleic acid vaccines reported progress in enhancing immunogenicity. The authors reviewed advances in providing protection during the delivery process and specific targeting to immune tissues, developing biomaterials to act as co-stimulators for vaccines (for example, taking advantage of 'tunable mechanical properties and adjusted multifunction for combining with other co-stimulators, such as CpG and cytokines'), and providing 'sustained release of nucleic acid antigens, which will establish the immunological memory for prolonged surveillance against pathogens or cancer cells'. The authors also note 'a lack of deep and comprehensive understanding the *in vivo* behavior of delivery vectors and immunostimulative mechanisms. This impedes progress towards the rational design of such delivery systems they argue.

352 In August 2015, Galanie *et al* reported having designed and engineered yeast to produce the selected opioid compounds thebaine and hydrocodone starting from sugar. The authors used enzyme discovery, enzyme engineering, and pathway and strain optimization techniques. The engineered pathway required "the expression of 21 (thebaine) and 23 (hydrocodone) enzyme activities from plants, mammals, bacteria, and yeast itself". The authors noted that this "is a proof of principle, and major hurdles remain before optimization and scale-up could be achieved. Open discussions of options for governing this technology are also needed in order to responsibly realize alternative supplies for these medically relevant compounds".

353 In May 2015, Mitchell *et al* reported having developed a qPCR-based high-throughput approach for the detection of the specific tags being engineered into the synthetic yeast being developed by an international consortium. The authors reported a much improved method for being able to differentiate between natural and synthetic yeast.

354 In March 2014, Lin *et al* reported a novel method for the efficient *in vivo* assembly of designed DNA fragments up to 10 kb long in Saccharomyces cerevisiae. The new method "reduces the time-consuming and labour-intensive efforts of yeast assembly by improving the screening efficiency for correct assemblies". The method is also relatively simple, the paper describes its use by undergraduates. The authors conclude that the method "may find routine applications in the construction of DNA fragments, especially in hierarchical assembly projects". It is already in use in the SC2.0 synthetic yeast project.

355 In July 2015, Mitchell *et al* reported a novel method for compiling genetic pathways for expression in Saccharomyces cerevisiae. It makes use of the yeast's capacity for homologous recombination to join sequences together. The authors used the new method to compile four, five and six gene pathways to produce β-carotene and violacein.

356 In October 2011, Conrado *et al* reported having used DNA to guide assembly of components along a biosynthetic pathway. The authors demonstrated that by controlling the special/temporal space in which these pathways work titres of the end product increases. They demonstrated raising titres for a number of metabolic products, including resveratrol, 1,2-propanediol, and mevalonate.

357 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted the 'development of a synthetic approach that rapidly generated influenza vaccine viruses from sequence data'. The authors noted 'DNA nanostructures were used as scaffolds to assemble antigen-adjuvant complexes that elicited a strong and specific antibody response without inducing a reaction to the DNA nanostructure itself'.

358 A June 2014 review of the implications of the convergence of biology and chemistry highlighted 'for chemicals produced by multi-enzyme processes, the physical spatial organisation of enzymes has been demonstrated to increase the efficacy and yield. Such approaches have also enabled the tuneable adaptation of metabolic flux. Proteinaceous and DNA-based scaffolds have been used to spatially locate enzymes important for the production of discrete and complex chemicals. These scaffolds have been developed for use in bacteria and yeast used for industrial production'.

359 A May 2012 review of plant-based production highlighted that the first biological drug receiving regulatory approval for use in humans. The drug, Protalix's Elelyso, was manufactured inside modified plant cells. The authors noted that at least four other drugs, including a H5N1 influenza vaccine, manufactured in plant-based systems were in the pipeline.

360 In May 2012, Jul-Larsen *et al* reported having produced a vaccine candidate for H1N1 influenza virus in recombinant plants. The authors used 'a recombinant influenza haemagglutinin antigen (HAC1) that was derived from the 2009 pandemic H1N1 (pdmH1N1) virus and expressed in tobacco plants'. They demonstrated that 'the tobacco derived recombinant HAC1 antigen is a promising vaccine candidate recognized by both B- and T cells'.

361 In October 2012, Olinger *et al* reported having produced monoclonal antibodies to treat Ebola in plant cells. The authors demonstrated that they protected rhesus macaques from lethal challenge when administered 1 h post-infection.

362 A November 2014 ECRI Institute review highlighted that a trivalent monoclonal antibody therapy for Ebola is 'humanized and recombinantly manufactured in the tobacco plant Nicotiana benthamiana'. The authors describe the steps involved:

- 'Planting Nicotiana seeds in flats
- Growing for several weeks with careful control of light, temperature, and humidity
- Treating plants with the antibody vector system
- Growing for an additional week allowing the plants to manufacture humanized antibody proteins
- Harvesting and homogenizing the humanized antibody proteins in a large vat followed by separation of the antibody proteins using a series of specialized purification techniques
- Testing the resultant antibody for purity and potency'.

363 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted 'the widening diversity of vaccine production methods now include… transgenic animals and plants'. The authors noted:

- 'Recombinant proteins have been produced in the milk of transgenic animals';
- 'Work has recently been revived… on the production of malaria antigens as potential vaccine candidates in the milk of transgenic goats'
- 'Antigens from several human and veterinary pathogens have been expressed in transgenic plants, e.g. rabies, hepatitis B, measles, avian influenza and anthrax';
- 'A vaccine candidate based on transient expression of the recombinant protein antigen in the leaves of the tobacco plant, which is then extracted and purified to obtain clinical grade material, is in clinical trials';
- 'There have been many reports on studies on the use of transgenic plants for the production of veterinary vaccines… One recent example is the development of transgenic alfalfa plants expressing a recombinant antigen that could induce neutralising antibodies to bovine viral diarrhoea virus'.

364 A July 2012 presentation by Pitt to the BWC Meeting of Experts highlighted a range of advances and technologies in the life sciences, including:

- 'significant advances in the bulk production of biological agents';

- ‘advances in the delivery of biological agents (peptides, proteins, DNA) to biological systems (usually based on nanotechnology);
- Effective protein engineering leading to designer proteins and protein binding agents, and ‘New detection technologies (e.g. peptide aptamers, SOMAmers, etc.)’;
- Nanomaterial and nanoparticle technologies for targeted delivery of chemical and biological agents;
- Smart delivery including: self assembling (and disassembling) systems; “responsive” to environment; transdermal and aerosol delivery; and neurological targeting;
- Synthetic biology, being used to engineer bacteria, yeast, insects and plants; and
- Distributed computing technologies to address bioinformatics and big data challenges.

365 In October 2013, Gomila *et al* reported ‘that the use of chimeric HA and NA segments with high-growth backbones is a viable strategy that could improve influenza vaccine manufacturing’. The authors demonstrated that three optimized backbones could be further improved by the inclusion of chimeric hemagglutinin (HA) and neuraminidase (NA) genome segments.

366 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted a number of developments in vaccine delivery, including:

- ‘Non-parenteral routes of administration, such as intranasal, aerosol, oral and transcutaneous are also being exploited’;
- ‘A novel vaccination concept that uses gold nanoparticles as a delivery vehicle for presentation of viral antigens to induce a protective immune response’;
- ‘The development of nanotechnology-based skin patches has offered a potential alternative for vaccine delivery to overcome some of the disadvantages of needles and syringe’;
- ‘Another means that has been proposed to address the limitations of the refrigeration requirement for distribution and storage is the stabilisation of vaccines in silk protein’.

367 In July 2012, Zhang *et al* reported having used self-standing silk protein biomaterial matrices to stabilize labile vaccines and antibiotics up to temperature of 60’c for over 6 months. The authors note that such an approach negates the need for a cold chain for such drugs.

368 In April 2013, Wang *et al* reported rationally integrating biomimetic nucleating peptides onto the capsid of enterovirus type 71 by reverse genetics. As a result, ‘calcium phosphate mineralization can be biologically induced onto vaccine surfaces under physiological conditions, generating a mineral exterior’. The authors note that they have improved ‘the thermostability and immunogenicity of live vaccines by self-biomineralization’. The demonstrated ‘t he self-biomineralized vaccine can be stored at 26 °C for more than 9 d and at 37 °C for approximately 1 wk. Both in vitro and *in vivo* experiments demonstrate that this engineered vaccine can be used efficiently after heat treatment or ambient temperature storage’.

369 An October 2014 review of the impacts of convergence of chemistry and biology highlighted the potential to use patchy particle nanoparticles ‘to better understand the underlying causes of the thermal instability of vaccines in order to find ways of stabilizing them for transport (for example by adding sucrose to increase the viscosity so as to prevent small DNA releases), which practically speaking could potentially revolutionize the laborious logistical process of vaccine delivery’.

370 In August 2011, Siegwart *et al* reported having developed tools to tune the cores and shells of nanoparticles for intercellular delivery. The authors reported having conducted a ‘high-throughput study of 1,536 structurally distinct nanoparticles with cationic cores and variable shells’. This ‘revealed structure-function relationships and beneficial design guidelines’.

371 In October 2011, Chung *et al* reported having developed biomimetic self-templating supramolecular structures. The authors noted that their ‘approach provides insight into the complexities of hierarchical assembly in nature and could be expanded to other chiral molecules to engineer sophisticated functional helical-twisted structures’.

372 A May 2012 review by Couvreur of the use of nanopartices in drug delivery highlighted that ‘nanoparticle suspensions which contain medicines i.e. ‘nanomedicines’) has made it possible to increase the therapeutic index of many components (improvement of the activity, reduction of toxicity) by selectively directing them towards the diseased tissues and cells (‘drug targeting’)’. The author also notes that ‘the shift in size from tens of micrometers to tens or hundreds of nanometers has thus been a significant technological and medical breakthrough’. They list a limited number of nanocarriers that were already on the market or in phase III trials. The author highlights progress towards improving drug loading and timed release, in particular developments in ‘“squalenoylation technology” or the construction of nano metal oxide frameworks’.

373 A December 2013 review by Zhao *et al* of the use of nanoparticles to deliver vaccines noted significant progress. The authors reported that the use of nanoparticles in vaccine formulations can improve antigen stability and immunogenicity and enable targeted delivery and slow release. They note that ‘a number of nanoparticle vaccines varying in composition, size, shape and surface properties have been approved for human use and the number of candidates in increasing’. The authors also note that there remains a need for additional understanding of mechanisms of action, biodistribution and the fate of particles is required before full rational design of such particles in feasible.

374 A June 2014 review by the OPCW of the implications of the convergence of biology and chemistry highlighted:

- ‘The shortcomings of peptides as drugs (and by implication for uses prohibited by the Convention) can be moderated in several ways. Formulations, particularly associated with liposomes or nanocarriers, are being explored to enhance penetration of the blood brain barrier, overcome host defences, and target specific organs’;

- ‘Nanoparticle-based formulations are being widely explored for enhanced or ‘smart’ drug delivery. Examples are controlled drug release, enhanced penetration of the blood brain barrier (e.g. for therapeutic peptides), and targeting specific organs or cells (e.g. cancer cells). Nanoparticles most commonly used in drug formulations include: imprinted polymers, dendrimers, vesicles, nanospheres, nano-capsules, micelles, carbon nano-tubes, liposomes, and nano-emulsions. Additional bio-based nanocarriers are being researched including DNA-based systems and viral-based systems’;
- ‘Nanocarrier-based delivery systems present several advantages over the classic ones: overcoming solubility problems, protecting the drug from the external environment (temperature, UV radiations, pH), and controlling the release profile’;
- ‘Nanocarrier-based delivery systems permit a more precise and controlled targeting at the site of action, while reducing the time of exposure at non-targeted tissues. This can increase efficacy, and reduce toxicity and side effects’.

375 In June 2012, Yen-Ting *et al* reported the structure of a self-assembling, 12 sub-unit protein cage. The authors note their approach ‘of fusing together oligomeric protein domains can be generalized to produce other kinds of cages or extended materials’.

376 A July 2012 presentation by Pitt to the BWC Meeting of Experts highlighted a range of advances and technologies in the life sciences, including:

- ‘Advances in the delivery of biological agents (peptides, proteins, DNA) to biological systems (usually based on nanotechnology);
- Nanomaterial and nanoparticle technologies for targeted delivery of chemical and biological agents;
- Smart delivery including: self-assembling (and disassembling) systems; “responsive” to environment; transdermal and aerosol delivery; and neurological targeting.

377 In October 2012, Zhao *et al* reported using DNA-based nanostructures to form ‘complex assemblies that co-localize drugs, targeting ligands and other functionalities in one nanostructure’. The authors used these structures to deliver the anthracycline drug duxorubcin (Dox) to three different human breast cancer cell lines. They demonstrated an ability to manipulate the design ‘to (i) tune the encapsulation efficiency and the release rate of the drug and (ii) increase the cytotoxicity and lower the intracellular elimination rate when compared to free Dox’.

378 In July 2015, Benson *et al* reported ‘a general method of folding arbitrary polygonal digital meshes in DNA that readily produces structures that would be very difficult to realize using previous approaches’. The authors noted their design process was highly automated and that the resulting structures ‘have a more open conformation… and are therefore stable under the ionic conditions usually used in biological assays’.

379 In December 2013, Huert *et al* reported having developed a computational framework for identifying design guidelines to increase the penetration of targeted nanoparticles into tumors. The models simulates the potency, motion, binding kinetics, and cellular internalization of targeted nanoparticles in a section of tumor tissue. The authors used the model to demonstrate the benefit of delaying nanoparticle binding until after the nanoparticles have had time to diffuse deep into the tissue. They concluded that following their design rules results in nanoparticles which “do not require fine-tuning of their kinetics or size and can be administered in lower doses than classical targeted nanoparticles for a desired tissue penetration”.

380 In July 2012, Zheng *et al* reported using Lipid-based liquid crystalline nanoparticles as novel drug-delivery systems for improving the bioavailability of both hydrophilic and hydrophobic drugs. The authors demonstrated that deliver using these particles led to a 2.1 times increase of the oral bioavailability of a drug.

381 In January 2013, Verma *et al* reported having used a magnetic core-shell nanoparticles for aerosol drug delivery. The authors demonstrated the feasibility of filling the nanoparticle with an anti-cancer drug and evaluated its delivery via aerosol administration.

382 In February 2013, Dian *et al* reported using a cubic phase nanoparticle for sustained release of ibuprofen. The authors demonstrated encapsulation efficiency greater than 85%, that in vitro release of the drug was greater than 80% at 24 hours, showing sustained release, and improved absorption compared to that of pure ibuprofen, with evidence of a longer half-life and a relative oral bioavailability of 222%.

383 In February 2013, Jin *et al* reported having used self-assembled liquid crystalline nanoparticles to overcome the low oral absorption and the rapid metabolization of an anti-cancer drug. The authors demonstrated the key indicators of bioavailability of the drug rose by 166% and 248% with the use of the nanoparticle.

384 In October 2014, Maddalo *et al* reported having used a viral delivery platform for CRISPR/CAS9 genome editing to deliberately create a cancer in a mouse lung. The authors report having used this genome editing technique to have improved the efficacy of the mouse model for studying human diseases.

385 In July 2015, Zinn *et al* reported having synthesised ancestral viral structures of Adeno-associated virus (AAV) vectors. These viruses have been used as delivery devices for gene therapy but ‘efforts to engineer AAV vectors have been hampered by a limited understanding of the structure-function relationship’. The authors characterised the resurrected viruses and identified one as ‘a highly potent *in vivo* gene therapy vector for targeting liver, muscle, and retina’.

386 In December 2012, DeMuth *et al* reported in Nature a novel delivery strategy for DNA vaccines, immune-stimulatory RNA, and biodegradable polycations. The authors used microneedles coated with releasable polyelectrolyte multilayers to deliver such agents into the epidermis. Following such application, such tattoos ‘promoted local transfection and controlled the persistence of DNA and adjuvants in the skin from days to weeks, with kinetics determined by the film composition’.

387 A March 2014 review of skin permeabilization for transdermal drug delivery identified a number of successful approaches, including: low-frequency sonophoresis, microneedles, electroporation and iontophoresis, and combinations of these methods. The authors noted that not all permeabilization methods are appropriate for all applications but that vaccination, protein delivery and analyte sensing are areas of impact. They noted the need for focused studies into applications utilizing the advantages of each method.

388 A May 2015 review by Lutton *et al* of the mechanical tests and insertion analytical techniques used by various groups to characterise microneedles highlighted 'the urgent need for consistency across the range of microneedle systems in order to promote innovation and the successful commercialisation of microneedle products'.

389 In May 2015, Vinayakumar *et al* reported development of a cup-shaped microneedle. The authors demonstrated 'reduced drug leakage resulting in improvement of efficiency of drug delivery and possibility of introducing multiple drugs'.

390 In July 2013, DeMuth *et al* reported that reproducing the kinetics profiles present during acute natural infections can improve the efficacy of vaccination with microneedles. The authors demonstrated that such an approach, relative to traditional parenteral needle-based immunization, led to a 10-fold increase in antigen-specific T-cell and humoral immune responses prompted by a model whole-protein vaccine.

391 A September 2013 review of microneedle patches for vaccine delivery noted that studies had demonstrated 'comparable or higher immunogenicity to conventional intramuscular routes, overall level of stability, and dose-sparing advantages. Furthermore, recent mechanism studies have begun to successfully elucidate the biological mechanisms behind microneedle vaccination'.

392 In June 2015, Yang *et al* reported having used a phase-transition microneedle patch for the trans-dermal delivery of insulin. The authors demonstrate only a minor loss of functionality in comparison to traditional injector pens with advantages for painless administration, freedom of refrigeration, and minimal safety concerns. They note the potential to use this delivery system for protein/peptide medicines requiring frequent dosing.

393 A January 2014 review by Okuda *et al* of developments to address the weak immunogenicity of DNA vaccines in humans identified a number of promising approaches, including: 'various genetic adjuvants, electroporation, and prime-boost methods [which] have been developed preclinically'.

394 In March 2015, van de Wal *et al* reported having used a tattoo delivery patch to administer a tumor vaccine. The authors demonstrated that despite a 10-fold lower overall antigen expression at the site of administration and draining lymph nodes when compared to intramuscular injection, tattoo injection resulted in higher or equal levels of immune responses.

395 In March 2015, Edens *et al* reported having developed and used microneedle patches for the delivery of inactivated polio vaccine. The authors demonstrated that these patches produced equivalent neutralizing antibody titres in monkeys when compared to traditional intramuscular injection. The noted several advantages including that they are 'easy to administer, have a small package size, generate no sharps waste and are inexpensive to manufacture'.

396 A July 2014 review of fabrication techniques for microneedles noted 'recent research has been conducted integrating various fabrication techniques for generating sophisticated microneedle devices for transdermal delivery including progress on their commercialization'.

397 A November 2013 review of the use of nanotechnology in biology and medicine surveyed nanotechnology-based approaches for precisely measuring and perturbing living systems. The authors noted that nanotechnology can be used "to characterize single molecules or cells at extraordinarily high throughput and deliver therapeutic payloads to specific locations as well as exhibit dynamic biomimetic behaviour". They noted particular applications for systems biology as well as new therapeutic strategies for personalized medicine.

398 An October 2014 review of the impacts of convergence of chemistry and biology (Speiz Convergence) report highlighted:

- 'Antibody-Drug Conjugates (ADCs) have been developed and tested for the targeted delivery of cytotoxic payloads to cancer cells… an approach has been developed which combines selectivity with toxicity, by linking antibodies and cytotoxic molecules together in the form of ADCs'. The authors note that 'at this stage of development, ADCs are expensive drugs, but the approach is considered competitive based on their increased efficacy. Moreover, two such approaches have met with FDA approval and some 30 other ADCs are currently in clinical trials at different phases. The Swiss company Lonza, for example, has developed both the capacity to produce bulk quantities and the specialist skills and experience in both the chemical and biotech fields necessary to manufacture ADCs. In terms of capacity, reactor sizes can vary from small (6 to 60 litres) to large scale (up to 600 litres, batch sizes up to 3 kg)';
- 'Nanoparticles, specifically… gold particles coated with hydrophobic and hydrophilic compounds could be used to imitate certain biological functions…. these nanoparticles have been shown to have the ability to penetrate cell membranes. Such nanoparticles could either be designed to carry small payloads of highly active drugs (for example certain peptides), or the nanoparticle itself could act as a drug by interacting with viruses'.

399 An April 2015 review by Cheng *et al* of targeting disease with polymeric nanoparticles notes 'the route of administration, molecular characteristics and temporal control of the nanoparticles are potential design variables that must be considered simultaneously' and highlighted the importance of such a holistic approach to facilitate the clinical applications of the technology.

400 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'Human genome mapping, studies on how the polymorphism in the human genes is linked to some causative agents of human diseases, the advancement of molecular and cellular biology, the knowledge of genetic features of different races and nationalities allow for possibilities in principle to manipulate with human genome and to selective impact on certain races'.

401 An August 2012 review of nanotechnologies in therapeutics highlighted targeting strategies, including both active and passive targeting, and factors impacting drug delivery, including the size and shape of nanoparticles and their surface characteristics.

402 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted:

- 'The production of toxic chemicals, including toxins, through biological synthesis (an aspect of the convergence of chemistry and biology); encapsulation and delivery through nanotechnology; and flow microreactors, which enable types of chemical reactions under conditions that were not previously technically feasible';
- 'The application of nanotechnology to improve materials and systems relevant for protection against CW and chemical decontamination is an area of gradual, but important development. Some areas of ongoing research that should continue to be monitored include the development of nanofibers for protective clothing, nanocatalysts for decontamination methods, and nano-based drug delivery mechanisms for medical countermeasures'; and
- 'New polymers, lipids, and other carrier materials for drugs or genes is an area of active research. These carriers may be used as components of nanomedicines, in which the properties of the carrier influence the biodistribution of the therapeutic agent that is bound or complexed with it. Both the chemical properties of the carrier (e.g., the relative hydrophilicity and hydrophobicity or the conjugation of "functionalizing" molecules to target particular cellular receptors) as well as physical properties (such as size and shape) influence the uptake and residence time of a drug in the body. These types of nanomedicines have a wide range of applications, including improved delivery across biological barriers such as the blood–brain barrier, prolonged residence time in the blood (reduced clearance in the liver), and improved targeting to particular cell types'.

403 A November 2013 review of emerging nanotechnology-based tools for biology and medicine, highlighted in developing nanocariers to deliver drugs. The authors noted advances to 'incorporate advanced functionality such as targeting to specific organs and cells, responsiveness to external stimuli, imaging capabilities, and drug delivery'. They provided an example of a nanoparticle which 'incorporates a ''stealth'' polymer brush shell that limits immunosurveilance as well as a degradable hydrophobic core that could be loaded with drugs. These optimized characteristics promoted circulation times while reducing liver accumulation'.

404 In May 2011, von Maltzahn *et al* reported the *in vivo* use of 'communicating' nanoparticles to increase tumor targeting. The authors used nanotechnology and synthetic biology approaches to construct systems with 'signalling' modules that "target tumours and then locally activate the coagulation cascade to broadcast tumour location to clot-targeted 'receiving' nanoparticles in circulation that carry a diagnostic or therapeutic cargo, thereby amplifying their delivery". The authors demonstrated the use of different types of signalling and receiving modules, transmitting information through several molecular pathways to increase targeting by over 40 times.

405 An August 2012 review of nanotechnologies in therapeutics highlighted:

- The use of nanocarriers and their application. The authors noted: nanocrystaline drugs including immunosuppressives, anti-emetics, hypercholesterolemia, and anti-anorexia; liposomes, including to treat fungal infections, ovarian and breast cancer, lymphomatous meningitis, and Kaposi's sarcoma; polymer-drug conjugates, including to treat enzyme deficiencies, leukaemia, and Hepatitis C; polymeric micelles for cancer chemotherapy; protein nanoparticles, to treat breast cancer; and lipid colloidal dispersion to treat fungal infections;
- Targeting strategies, including both active and passive targeting;
- Factors impacting drug delivery, including the size and shape of nanoparticles and their surface characteristics.

406 An October 2012 review by Zhang *et al* of advanced materials and processing for drug delivery highlighted 'innovation in material chemistry allows the generation of biodegradable, biocompatible, environment-responsive, and targeted delivery systems. Nanotechnology enables control over size, shape and multi-functionality of particulate drug delivery systems'.

407 A July 2011 review by Yoo *et al* of synthetic carriers, such as polymer and lipid particles, noted that they often fail to meet clinical expectations. The authors highlight progress in re-engineering the various natural carriers which are 'highly optimized for their specific functions *in vivo* and possess features that are often desired in drug delivery carriers'.

408 An April 2014 review of advanced drug delivery systems highlighted that 'intelligent drug delivery systems are continuously improved with the purpose to maximize therapeutic activity and to minimize undesirable side-effects'. The authors noted 'advanced drug delivery systems based on micelles, polymeric nanoparticles, and dendrimers' and that 'polymeric carbon nanotubes and many others demonstrate a broad variety of useful properties'.

409 In March 2012, Tong *et al* reported having developed a new type of nanoparticle drug delivery system. The authors demonstrate that the particle reversibly changes shape and size when stimulated by UV light allowing spatiotemporal control of drug release and allowing for 'repetitive dosing from a single administration'. The authors also demonstrate that the nanoparticle facilitates tissue penetration.

410 In July 2012, Zheng *et al* reported having developed a nanocarrier capable of crossing the epidermal barrier to deliver siRNA. The carrier 'can be delivered in a commercial moisturizer or phosphate-buffered saline, and do not require barrier disruption or transfection agents'. The authors note that the delivery devices 'are virtually undetectable in internal organs'.

411 A July 2012 presentation by Japan to the BWC Meeting of Experts highlighted the dual use potential of recent developments, including in:

- Nanobiology providing capabilities to engineer an 'artificial membrane to cover disease-causing agents as well as toxins'; and
- Nanotechnology drug delivery devices which enhance the 'infectious ability of microorganism' and delivers drugs across the blood brain barrier.

412 In April 2013 Impel NeuroPharma announced 'the first ever successful neuroimaging study demonstrating peptide delivery direct to the brain'. The study made use of the company's precision olfactory delivery devices that increase drug deposition to the upper nasal cavity from around 5% to greater than 50%.

413 A December 2013 review by Zhao *et al* of the use of nanoparticles to deliver vaccines noted significant progress. The authors reported that the use of nanoparticles in vaccine formulations can improve antigen stability and immunogenicity and enable targeted delivery and slow release. They note that 'a number of nanoparticle vaccines varying in composition, size, shape and surface properties have been approved for human use and the number of candidates in increasing'. The authors also note that there remains a need for additional understanding of mechanisms of action, biodistribution and the fate of particles is required before full rational design of such particles in feasible.

414 In March 2012, Schroeder *et al* reported having developed a remotely activated protein-producing nanoparticle. The authors housed components of cellular machinery necessary for transcription and translation of Green Fluorescent Protein and enzymically active luciferase inside of a lipid vesicle. The authors demonstrated spatiotemporal control of protein production in vitro and *in vivo* by 'irradiating micrometer-scale regions on the time scale of milliseconds'.

415 In October 2011, Singh *et al* reported having developed bioresponsive nanoparticles for drug release. The authors designed and built mesoporous silica nanoparticles with therapeutics loaded into core and shell domains. The authors demonstrated in vitro that in response to proteases found at tumor sites, the nanoparticles released their payload and were capable of inducing cellular apoptosis.

416 In September 2013, Dapat *et al* reported on sensitivity in Japan to the antiviral Neuraminidase inhibitors used to treat influenza. The authors noted that there had been a rise in resistant viruses but that prevalence remained low. During the 2010-2011 influenza season, viruses were circulating with a 200-300-fold increase in resistance to two of the four licensed Neuraminidase inhibitors – one of which had only been licensed the same year. A similar paper from January 2014 reviewed the susceptibility of influenza viruses circulating in the United States from 2011 to 2013 to all four Neuraminidase inhibitors. The authors noted that resistance was only identified in a single sample, which included rare mutations reducing inhibition by oseltamivir (31-fold) and zanamivir (66-fold). A March 2014 paper examining resistance to the two most recently licensed Neuraminidase inhibitors in samples collected from Asia, Africa and Oceania revealed no resistance to one and 3.2% resistance in Influenza A viruses.

417 In July 2014, Ashley *et al* reported that P. falciparum resistant to artemisinin was prevalent across mainland Southeast Asia. The authors noted that the drug resistance is associated with mutations in kelch13. The authors demonstrated that 'prolonged courses of artemisinin-based combination therapies [comprised of artesunate followed by a standard course of artemisinin] are currently efficacious in areas where standard 3-day treatments are failing'.

418 In May 2013, Miotto *et al* reported the identification of subspecies of P.falciparum exhibiting resistance to artemisinin in Cambodia. The authors cataloged SNPs connected to resistance, including coding variants in transporter proteins and DNA mismatch repair proteins.

419 A May 2012 review of Carbapenem resistance in *Enterobacteriaceae* highlighted worldwide emergence of resistance not only in hospital settings but also in the community. The authors noted the 'corresponding genes are mostly plasmid-located and associated with various mobile genetic structures (insertion sequences, interons, transposons), further enhancing their spread'. They noted progress in understanding related activity, distribution, clinical impact, and possible novel antibiotic pathways.

420 In March 2014, Fowler and Hanson reported the specific mutation in Pseudomonas aeruginosa that confers carbapenem resistance. The authors demonstrated that in each of the isolates they sequenced the location of the insertion was different.

421 In May 2014, Adler reported the mechanisms and dynamics of carbapenem resistance in *Escherichia coli*.

422 In February 2015, Witchuda *et al* reported Molecular Epidemiology and Mechanisms of Carbapenem Resistance of Acinetobacter spp. . The authors reported the specific gene insertions necessary to confer resistance.

423 A January 2013 review of mechanisms of resistance to the four licensed neuraminidase inhibitors used to treat and prevent influenza highlighted that 'because of differences in their chemistry and subtle differences in NA structures, resistance can be both NAI- and subtype specific'. The author identifies a number of specific mutations that confer anti-viral resistance or reduced sensitivity.

424 In May 2012, Kos *et al* reported the mutation responsible for vancomycin resistance in *Staphylococcus aureus*. The authors demonstrated that in all of the cases in the US to date, the bacteria had all spontaneously evolved a mutation residing on a fusion of plasmids from S. aureus and an enterococcal bacteria. By July 2013, VRSA had spread to Europe.

425 In April 2014, Rossi *et al* reported a case of transferable Vancomycin Resistance in Stapholococcus aureus.

426 In July 2014, Gardete and Tomasz reported having developed a model that describes the mechanisms of vancomycin resistance in *Staphylococcus aureus*.

427 In January 2014, Renzette *et al* reported how the genome of Influenza A viruses evolve during the development of oseltamivir resistance in vitro. The authors demonstrate that 'only selection of H274Y is required for oseltamivir resistance and that H274Y is not deleterious in the absence of the drug'.

428 An October 2012 review of biofilm infections highlighted that major regulators of biofilm formation are associated with antibiotic tolerance and that different components of these signalling networks might be appropriate targets for antibiofilm therapy in combination with antibiotic treatment strategies'. The authors noted that given the 'almost universal presence' of microbial-associated molecular patterns, conserved structures 'are also potential immunotherapeutic agents to treat antibiotic-resistant bacterial infections'.

429 In January 2013, Wang *et al* reported the structural mechanism through which a mutation confers resistance to influenza drugs that target the M2 ion channel (amantadine and rimantadine). The authors identified small molecule drugs that work in a similar manner to amantadine and rimantadine, are not affected by the drug resistance mutation and show greater potency. Additional details of these potential new drugs were published in April 2013.

430 In April 2014, Polishchuk *et al* reported the thermodynamic and functional characterization of the mutation that makes influenza A viruses resistant to drugs targeting their M2 ion channel.

431 In December 2014, Plug *et al* reported the structure of Influenza A polymerase bound to the viral RNA promoter. The authors asserted that 'this structure lays the basis for an atomic-level mechanistic understanding of the many functions of influenza polymerase, and opens new opportunities for anti-influenza drug design'.

432 In January 2015, Mok *et al* reported having used population transcriptomics of human malaria parasites to reveal the mechanism of artemisinin resistance.

433 A February 2013 review of bacterial populations responses to non-lethal concentrations of antibiotics acknowledged that 'bacteria may sense antibiotics as extracellular chemicals to trigger different cellular responses, which may include an altered antibiotic resistance/tolerance profile'. The authors discussed 'the different types of interactions mediated by antibiotics and non-antibiotic metabolites as a function of their concentrations and speculate on how these may amplify the overall antibiotic resistance/tolerance and the spread of antibiotic resistance determinants in a context of polymicrobial community'.

434 In July 2012, Grant *et al* reported the role of persister cells in infections with Mycobacteria. The authors demonstrated that 'that the small persister subpopulation within a larger antibiotic-susceptible population also shows differential susceptibility to antibiotic-induced hydroxyl radicals. Furthermore, we show that stimulating ROS production can eradicate persisters, thus providing a potential strategy to managing persistent infections'.

435 In July 2013, Orman and Brynildsen reported that dormancy is not necessary or sufficient for bacterial persistence and that a range of additional characteristics is needed to define the persister phenotype.

436 In March 2014, Amato and Brynildsen reported that nutrient transitions are a source of persisters in *Escherichia coli* biofilms.

437 In March 2012, Vega *et al* reported that bacterial populations use Indol-activated genes that respond to stress to signal a sub-set the population to enter into a dormant state. That dormant state reduces the impact of antibiotics, allowing them to tolerate higher levels. The identification of the importance of these persister cells and the signalling molecules that prompt their formation offers a novel target for efforts to reduce antibiotic resistance.

438 In February 2014, Butt *et al* described part of the mechanism controlling persistors in Burkholderia pseudomallei populations.

439 In February 2014, Ghorbal *et al* reported having used the CRISPR/CAS9 genome editing system in P. falciparum the causative agent of malaria. The authors demonstrated 'disrupting chromosomal loci and generating marker-free, single-nucleotide substitutions with high efficiency'. They used CRISPR/CAS9 to add polymorphisms known to confer resistance to artemisinin.

440 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'active studies in the field of *Enterobacteriaceae* (Salmonella, *Escherichia coli*), the most widespread group of microorganisms which inhabit the intestines of humans and animals and can contaminate the environment, food and ready meals and cause anthrax and cholera. In 2011-2012 a number of scientists published their findings on *Enterobacteriaceae*, Salmonella and anthrax (USA), anthrax (Japan), *Escherichia coli* (People's Republic of China) and cholera (Iran)... Study areas include both the genetic multidrug resistance mechanisms and the possibility of an artificial simulation of new strains resistant to certain groups of antibacterial agents'.

441 A July 2015 review of antibiotics currently in clinical development found that "an estimated 36 new antibiotics1 that have the potential to treat serious bacterial infections are in clinical development for the U.S. market. The success rate for drug development is low; at best, only 1 in 5 candidates that enter human testing will be approved for patients". The review, which had been updated periodically since February 2014 provided details of drugs in the pipeline, including the name, development phase, company, the type of drug, expected activity against resistant strains, expected activity against priority pathogens, and potential indication(s).

442 A December 2014 review of antibiotics currently under development estimated that 37 candidates were in clinical development. This number had fallen from 45 candidates in February 2014. The authors noted:

- 'Of the 37 antibiotics in development, 10 were in Phase 1 clinical trials, 18 in Phase 2, eight in Phase 3, and one has a new drug application submitted. Historically, about 60 percent of drugs that enter Phase 3 will be approved';
- 'At least two antibiotics in early development attack bacteria in an entirely new way by sidestepping the resistance of some bacteria to available antibiotics. Other drugs in the pipeline attack the same targets in bacteria as available drugs but seek to thwart resistance by using new chemical compounds'; and
- A shift in who was developing antibiotics – 'Nearly 80 percent of the products currently in development are being studied by small companies rather than the large pharmaceutical firms that once dominated this field.3 Additionally, just under half of the companies are considered pre-revenue, meaning that they have no products currently on the market'.

443 An April 2015 review of novel influenza therapies highlighted 'several new classes of antiviral candidates targeting viral replication through individual domains of the polymerase and the nucleoprotein have been developed through structure-based design'. The authors noted 'Antiviral candidates targeting the NP and polymerase domains are in the pipeline but their pharmacokinetics needs further studies. The recently published structures of the polymerase expand the possibilities for development of new antivirals'.

444 In July 2011, Rider *et al* reported having used a new approach to kill cells containing viral genetic material. The mechanism, 'dubbed Double-stranded RNA (dsRNA) Activated Caspase Oligomerizer (DRACO) that selectively induces apoptosis in cells containing viral dsRNA'. The authors 'created DRACOs and shown that they are nontoxic in 11 mammalian cell types and effective against 15 different viruses'. In March 2015, Guo *et al* reported using this system to inhibit Porcine Reproductive and Respiratory Syndrome virus replication in vitro.

445 In April 2014, Alhadeff *et al* reported computational and experimental analysis of drug binding to the Influenza M2 channel. The authors used a cell-based assay to identify two new Influenza M2 channel blockers, demonstrates that these were more effective than Rimantadine against otherwise resistant flu strains, and used computational analyses to provide insights into the increased efficacy of the new compounds.

446 In December 2014, Wu *et al* reported having identified a compound capable of blocking the M2 ion channel in Influenza A viruses in both its mutated and wild type. The authors modelled how the compound fits and binds to the ion channel in both cases, opening the door 'for the next round of rational design of broad-spectrum antiviral drugs'.

447 In January 2015, Zhao *et al* reported having identified a camphor derivative as novel M2 ion channel inhibitors of influenza A virus. The authors demonstrate that the new compound takes up more space in, thereby blocking more completely, the ion channel.

448 In April 2013, Vigant *et al* reported developing a non-cytotoxic, membrane-targeted, broad-spectrum antiviral which inhibits the entry of many lipid-enveloped viruses. The authors delineated the molecular mechanism which underlies antiviral activity and improved its photochemical, photophysical, and pharmacokinetic properties. The reported 'encouraging *in vivo* efficacy against a lethal emerging pathogen'.

449 In October 2013, Kessler *et al* reported the discovery and synthesis of novel benzofurazan derivatives which act as inhibitors of influenza A virus.

450 In November 2013, Furuta *et al* reported on a novel viral RNA polymerase inhibitor, Favipiravir. The drug had competed Phase II clinical trials for use against influenza viruses in USA and Japan. The authors noted that 'favipiravir blocks the replication of many other RNA viruses, including *arenaviruses*...; phleboviruses...; hantaviruses ...; *flaviviruses*...; enteroviruses...; an alphavirus...; a paramyxovirus...; and noroviruses'. Favipiravir has subsequently been used in clinical trials against Ebola Virus Disease.

451 In May 2014, Lepri *et al* reported having optimized small-molecule inhibitors of Influenza Virus Polymerase. These drugs target a new part of these viruses, potentially offering entirely new therapeutic options not currently impacted by drug resistance.

452 In May 2015, Massari *et al* reported having developed a hybrid small molecule disrupting the function of Influenza A polymerases. The authors demonstrated 'the identification of compounds endowed with both the ability to disrupt PA–PB1 subunits interaction and anti-Flu activity with no cytotoxicity'.

453 In December 2011, Okandeji *et al* reported having identified an enhanced compound capable of blocking efflux pumps in certain multidrug resistance bacteria making them susceptible again to antibiotics. Similar compounds have been used to this end in Gram-negative bacteria. The authors identified a related compound that worked in Gram-positive bacteria and used it to 'inhibit two chloramphenicol-specific efflux pumps in Streptomyces coelicolor, a Gram-positive bacterium that is a relative of the human pathogen *Mycobacterium tuberculosis*'. The authors synthesised a library of related compounds, one of which demonstrated three-fold efficiency improvements when compared to the wild type.

454 A May 2015 review of therapeutic options and emerging alternatives for multidrug resistant staphylococcal infections is a tour de force of recent developments to deal with bacterial infections. The authors highlighted:

- 'A range of approved antibiotics from the glycopeptide, lipopeptide, pleuromutilin, macrolide, oxazolidinone, lincosamide, aminoglycoside, tetracycline, steptogramin, and cephalosporin classes has been employed to treat MRSA infections.
- The upcoming pipeline of drugs for MRSA includes some new compounds from the above classes, together with fluoroquinolones, antibacterial peptide mimetics, aminomethylciclines, porphyrins, peptide deformylase inhibitors, oxadiazoles, and diaminopyrimidines.

- A range of non-drug alternative approaches has emerged for MRSA treatment. Bacteriophage-therapy including purified lysins has made a comeback after being discovered in the 1930s. Quorum-sensing inhibitors are under investigation. Small molecule inhibitors of multi-drug efflux pumps may potentiate existing antibiotics.
- The relative failure of staphylococcal vaccines is being revisited by efforts with multi-valent vaccines and improved adjuvants.
- Photodynamic therapy uses non-toxic photosensitizers and harmless visible light to produce reactive oxygen species that can nonspecifically destroy bacteria while preserving host cells.
- Preparation of nanoparticles can kill bacteria themselves, as well as improve the delivery of anti-bacterial drugs'.

455 In October 2012, Lin *et al* reported that a new class of antibiotics then under development, LpxC inhibitors, were able to disarm but not kill certain types of antibiotics resistant pathogens. The authors demonstrated that the new antibiotics 'blocked the ability of bacteria to activate the sepsis cascade, enhanced opsonophagocytic killing of the bacteria, and protected mice from lethal infection'.

456 In June 2013, Morones-Ramirez *et al* reported having identified the antibacterial properties of silver. The authors demonstrate that 'silver disrupts multiple bacterial cellular processes, including disulfide bond formation, metabolism, and iron homeostasis. These changes lead to increased production of reactive oxygen species and increased membrane permeability of Gram-negative bacteria that can potentiate the activity of a broad range of antibiotics against Gram-negative bacteria in different metabolic states, as well as restore antibiotic susceptibility to a resistant bacterial strain'. They demonstrated in vitro using silver: to increase the potency of antibiotics; to expand the antibacterial spectrum of antibiotics (sensitizing Gram-negative bacteria to the Gram-positive–specific antibiotic vancomycin); and in combination with antibiotics, to eradicate bacterial persister cells.

457 In July 2013, Jang *et al* reported having identified a novel antibiotic from a marine-derived Actinomycete. The authors demonstrated that 'chlorination of anthracimycin gives a dichloro derivative that retains activity against Gram-positive bacteria, such as anthrax, but also shows activity against selected Gram-negative bacteria'.

458 In May 2015, Kwan *et al* reported the FDA-approved anti-cancer drug mitomycin C eradicates persister cells. The authors demonstrated that the drug: eradicates cells grown in numerous different growth states and in both rich and minimal media; worked against persisters from multiples species of bacteria, including *Escherichia coli* K-12 as well as pathogenic species of E.coli, *Staphylococcus aureus* and Pseudomonas aeruginosa; and its efficacy in 'an animal model and a wound model, substantiating the clinical applicability of MMC against bacterial infections'.

459 A November 2011 review of best practice in improving community buy-in noted the importance of behavioural and social scientists in: helping communities to adopt proven interventions; and to scale up such measures to increase the impact of interventions. The author noted the importance of addressing cultural barriers from within communities.

460 In September 2014, the World Health Organization published details as to how anthropologists were helping medics fight Ebola Virus Disease in Guinea. In particular they noted: many villagers are wary of the medical teams battling the epidemic; anthropologists from the sub-region helped to mediate with local people; and that traditional beliefs and the high illiteracy rate make the task harder.

461 In January 2015, the World Health Organization published information on best practice learned from the Ebola Virus Control Teams deployed in West Africa. They noted the important role that anthropologists played in listening to affected communities, providing psychosocial assistance to families and people who have come into contact with the disease, and help to calm the fears of people. Such integration into the communities has helped identify additional cases of the disease and determine additional chains of infection, assisting in epidemiological and control efforts.

462 A December 2012 review of best practice in preventing pandemics via international development found that 'that the most prominent driver is the breakdown or lack of public health infrastructure and argue that there is a mismatch between the drivers of public health events and current trends in public health spending and pandemic prevention'.

463 An April 2013 review of prevention and control strategies for emerging infectious diseases highlighted that 'the response to pandemic influenza outbreaks has improved markedly in terms of control strategies, stockpiles of antivirals, and vaccine development. These improvements also suggest advances in disease surveillance, transparency in reporting, and regional collaboration and cooperation'. The authors noted shortcomings in focusing 'on high-risk groups, quantitative and measurable results… and quantitative assessment'.

464 In April 2013, Milne *et al* reported having modelled the cost effectiveness of pandemic influenza interventions. The authors demonstrated that 'the most cost effective strategies for mitigating an influenza pandemic involve combining sustained social distancing with the use of antiviral agents'. The authors also differentiated between low intensity and high intensity pandemics. The noted 'during low severity pandemics costs are dominated by productivity losses due to illness and social distancing interventions, while for high severity pandemics costs are dominated by hospitalisation costs and productivity losses due to death'.

465 In May 2014, Halder *et al* reported having modelled the cost effectiveness of pre-pandemic influenza vaccination. The authors demonstrate that 'compared to reactive vaccination, pre-emptive strategies would be more effective and more cost effective, conditional on the pre-pandemic vaccine being able to achieve a certain level of coverage and efficacy. Reactive vaccination strategies exist which are as effective at mortality reduction as pre-emptive strategies, though they are less cost effective'.

466 In March 2012, Hong *et al* reported an integrated model of environmental transport and human health exposure to biological pathogens. The authors used the model 'to develop quantitative guidelines for how environmental pathogen concentrations may be related to human health risk in an indoor environment'. The authors espouse the model to 'provide a framework for developing the many different environmental standards that are needed for making risk-informed response decisions, such as when prophylactic antibiotics should be distributed, and whether or not a contaminated area should be cleaned up'. This model was updated in May 2015 to take advantage of actual data from the sites of one of the anthrax letter attacks to reduce the uncertainties of inputs with uniformed prior estimates.

467 A January 2015 review of engineering control of respiratory infection and low-energy design of healthcare facilities highlighted 'active research and development on reducing hospital energy use while improving infection control and discusses the potential for conducting "clinical trials" to gain the necessary evidence to support changes in hospital ventilation design'.

468 In May 2015, Cooney *et al* reported on possibilities for community-based early detection for Ebola response. The authors use models to show that 50% engagement with such strategies could lead to a rapid decline of new cases. The also use the model to show 'travel restriction policies to be effective at reducing the risks associated with compliance substantially below the 40% level, shortening the outbreak and enabling efforts to be focused on affected areas'.

469 In May 2015, Hamilton *et al* reported 'an analysis of post-attack response strategies to mitigate the risks of reoccupying contaminated areas following a release of *Bacillus anthracis* spores… in an urban setting'. The authors determine that: for high-risk scenarios, evacuation and building decontamination is desirable; for medium-risk scenarios, 'the preferred option is to evacuate for a short period, vaccinate, and then reoccupy once the vaccine has taken effect'; for low-risk scenarios, only vaccination is necessary; and for minimal risk scenarios, no mitigation strategy offers any degree of cost effectiveness.

470 In March 2013, Milton *et al* reported that 'surgical masks worn by patients reduce aerosols shedding of virus. The abundance of viral copies in fine particle aerosols and evidence for their infectiousness suggests an important role in seasonal influenza transmission'.

471 A number of health agencies published advice on infection prevention and control measures for Ebola Virus Disease, following the largest ever outbreak of the disease in West Africa. For example, the Public Health Agency of Canada provided guidance on the minimum level of infection control measures, based on a review of available scientific evidence. The guidance covered: risk and transmission; preventing exposure and transmission; engineering controls, administrative controls and personal protective equipment; organizational risk assessment routine practices; point-of-care risk assessment; triage and screening; source control; hand hygiene; duration of precautions; notifications; handling of sharps; laboratory precautions; dedicated equipment; cleaning, disinfection and sterilization of medical equipment; environmental cleaning; handling bodies of deceased patients; handling dishes and cutlery; waste and linen management; education of patients and visitors; and visitor management.

472 A February 2013 review of the historical use of quarantine and other measures for controlling epidemic diseases highlighted controversies due to political, ethical, and socioeconomic issues and stressed the importance of finding a careful balance between public interest and individual rights.

473 A June 2014 review of the implications of the convergence of biology and chemistry highlighted 'new research approaches to physical protective equipment'. The authors noted 'Current research is directed primarily at systems which enhance protection but with reduced physiological burden for the wearer, and which are less cumbersome. There are also research efforts to develop self-decontaminating protective clothing, e.g. with the incorporation of enzymes and/or catalysts'. They highlighted research 'to develop nanomaterials as effective gas mask canister adsorbents and for protective clothing which may have a lower burden for the wearer than current systems'.

474 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted the application of nanotechnology to improve materials and systems relevant for protection against CW and chemical decontamination is an area of gradual, but important development. Some areas of ongoing research that should continue to be monitored include the development of nanofibers for protective clothing, nanocatalysts for decontamination methods, and nano-based drug delivery mechanisms for medical countermeasures'.

475 An October 2014 review of the impacts of convergence of chemistry and biology highlighted that international reviews of scientific and technological developments had shown that:

- 'the great benefits that convergence and, more generally speaking, the advances in the life sciences are expected to bring about. They reach from more effective medical countermeasures (for example… new diagnostic tools such as biosensors embedded in smart phones) to new decontamination methods or detectors for toxic chemicals as well as biological agents';
- 'advanced computational methods and directed evolution are used to develop enzymes tailored to address a specific industrial need… Given recent advances, it is now possible to take advantage of the tools of nature and those of molecular modelling to take existing, non-optimal enzymes and tailor their properties to drive chemical reactions with high selectivity and yield'. The authors noted that 'this work may be useful is in treatment or deactivation of harmful materials either *in vivo* or as part of decontamination'.

476 In July 2011, Calfee *et al* reported testing the efficacy of six sporicides in remediating contamination with *Bacillus anthracis* ames on a variety of outdoor surfaces. The authors demonstrated that non-porous surfaces were easily decontaminated but that porous surfaces presented more of a challenge. Only one sporicide decontaminated all surfaces.

477 In March 2012, Calfree *et al* reported testing the efficacy of three decontaminates in remediating contamination with *Brucella suis* on a variety of outdoor surfaces. The authors demonstrates that 'passive decontamination (through attenuation) may not be feasible, as this organism can persist for months. In addition, the results suggest that some sporicidal decontaminants may be ineffective on materials such as wood, even for vegetative biological agents such as *Br. suis*'.

478 In May 2012, Calfee *et al* reported evaluating the effectiveness of two spray-based decontamination methods for surface contamination reduction and to determine the potential for contamination spread by these methods. The authors demonstrated 'consideration of material surface type is important when selecting a decontaminant. Also, achieving conditions that effectively inactivate surface biological contamination are critical to preventing the spread of contamination'.

479 In January 2014, Kembel *et al* reported that architectural design drives the biogeography of indoor bacterial communities. The authors demonstrate 'that humans have a guiding impact on the microbial biodiversity in buildings, both indirectly through the effects of architectural design on microbial community structure, and more directly through the effects of human occupancy and use patterns on the microbes found in different spaces and space types. The impact of design decisions in structuring the indoor microbiome offers the possibility to use ecological knowledge to shape our buildings in a way that will select for an indoor microbiome that promotes our health and well-being'. Presumably, architectural decisions will also impact on how contaminated they might become following a deliberate release and how easily they can be decontaminated.

480 In January 2013, Yap *et al* reported data on the persistence of bacterial genomic DNA following autoclaving. They highlighted the potential for fragments of genetic material to be sufficiently complete to pose a risk for horizontal gene transfer – for bacterial species that come into contact with it to pick it up and to develop new properties. The authors highlight the potential for virulence genes, environmental persistence, or antibiotic resistance characteristics to spread in this manner. The results also offer interesting possibilities for downstream sampling (either in time or location) of effluent from facilities enabling inferences to be made as to what agents are being used, what characteristics those agents posses, and what activities were being undertaken. Equally, similar techniques might offer opportunities to find usable bioforensic data even after equipment has been autoclaved.

481 A 2015 review of the industrialisation of biology conducted by the US National Academies of sciences determined that "the past decade has seen an explosion in the technologies to com- pose, read, write, and debug DNA. This has rapidly increased the scale and sophistication of genetic engineering projects, and in the near term this will lead to more complex chemical structures and composite nano- materials, which require precise control over dozens of genes". The authors concluded that "lowered costs, increases in production speed, flexibility of manufacturing plants, and increased production capacity are among the many potential benefits that the increased industrialization of biology may bring to producers and consumers of chemical products that have not been previously available at scale". Furthermore "the growth of this field will enable the use of biology to produce high-valued chemical products that cannot be produced at high purity and high yield through traditional chemical synthesis. The future may also include a large number of high- volume chemicals, where biology represents a better synthetic pathway (cheaper and greener) than the conventional chemical synthesis".

482 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted a number of developments in vaccine production, including:

- The 'design of self-assembling influenza nanoparticle vaccines that elicit broader and more potent immunity than traditional influenza vaccines' using synthetic nanoparticles eliminating 'the need to grow potentially dangerous viruses in eggs or cell culture, a comparatively costly and time-consuming step of commercial vaccine production';
- 'Many developments in vaccine design result in elimination of the need for production in high containment facilities and reduction of manufacturing times';

483 An October 2014 review of the impacts of convergence of biology and chemistry highlighted that international reviews of scientific and technological developments had shown that genome editing techniques 'including zinc finger nucleases, CRISPR/Cas, TALEN, and meganuclease' are already in use. The authors noted 'one project of particular interest... is that of the use of gene editing to remove Ricin from the castor plant. Removal of the toxin would also have obvious positive implications for the control of Ricin'.

484 In August 2013, Langlois *et al* reported a molecular biocontainment approach that did not prevent influenza replication and transmissibility in ferrets, but did attenuate influenza pathogenicity in mice, and (based upon the presence of species-specific endogenous small RNAs) humans. Such an approach should address biosafety concerns around gain-of-function experiments and, according to the authors, 'should be applicable beyond influenza A virus to minimize the risk of experiments involving other pathogenic viruses'.

485 In December 2014, McNamara *et al* reported the 'rigorous screening program' developed by the International Genetically Engineered Machines Competition to screen projects for potential risks. The authors noted the procedures were designed 'to expand, not contract, the universe of acceptable projects'. They highlighted 'policy process evolution thus far, screening findings from the 2013 competition, and expectations for future policy evolution'

486 In April 2015, a desktop gene printer was released. The printer was capable of assembling up to 32 genes of between 400 and 1.8kb and included high fidelity production and dedicated error correction technologies.

487 A May 2014 review of emerging technology reported that two software engineers had compiled and rebooted a living virus from genetic material. The author noted that the two researchers working in an academic laboratory (to ensure the necessary safety and responsible conduct oversight) synthesised a bacteriophage in just over two weeks for about $1000. The genetic material was bought from commercial gene synthesis companies and then joined the fragments together and actived the virus – demonstrating that tacit knowledge is not a barrier to synthesing viruses.

488 A May 2014 review of large-scale de novo DNA synthesis highlighted that since the creation of infectious poliovirus in 2001, 'dozens of RNA viruses have been chemically reconstructed—including the 1918 Spanish influenza, the likely coronavirus progenitors to severe acute respiratory syndrome and many others for purposes of viral attenuation, historical reconstructions, vaccine development and viral genomic studies. Several DNA-based bacteriophages have been synthesized de novo as well. Beyond viral genomes, over a series of studies, the Venter Institute designed, built, assembled and transplanted a fully synthetic bacterial genome to encode a viable organism. Such efforts are only increasing. For example, the design, synthesis and viability of synthetically designed yeast chromosomal arms was shown… and work on a fully synthetic yeast genome is ongoing'.

489 A July 2012 working paper by the UK to the BWC Meeting of Experts highlighted 'small peptides, including toxins, can be chemically synthesised – a service that is now readily available commercially. As one recent study has noted, peptides are of interest in the incapacitating chemical agent context because peptide based bioregulators are responsible for the control of a number of vital physiological functions in the human body'.

490 An October 2014 review of the impacts of convergence of chemistry and biology highlighted that 'the technical capability for chemical synthesis of toxins exists, and synthetic biology approaches could also be used for toxin production and modification. Specifically, there is current research in synthesizing Saxitoxin by exploiting the function of sxt genes, expressing the Ricin A and B side chains through modifying E. coli, and chemically modifying conotoxins to develop new drug candidates'.

491 In December 2014, Jeon *et al* reported identifying genomic components that influence the toxicity of B. anthracis toxin through TNF-α production.

492 A May 2014 review of large-scale de novo DNA synthesis highlighted significant progress the understanding and engineering of regulatory elements. The authors noted:

- 'Recent efforts focused on understanding the structural and functional characteristics of thousands of cis-regulatory sequences governing transcriptional, translational and other regulatory processes in mammalian, yeast and bacterial systems. Over the coming years, NGS-based methodologies that are developed to measure transcription, translation, epigenetics, splicing and other gene regulatory phenomena will also be used to analyze synthetic libraries. The goal is to understand which sequences are responsible for causal changes to these processes and how we can use them to engineer new functionalities';
- To better understand and engineer particular genetic systems, agents have been refactored – their regulatory elements removed and well-characterized replacements added. Examples of refactoring in bacteria and bacteriophages were provided. 'The refactored system reconstituted functionality, albeit at reduced production levels. Improvements in the design and automated assembly of these refactored segments allowed reconstitution to wild-type production levels';
- Increasingly sophisticated methodologies for identifying and obtaining parts – for example, the application of 'synthetic metagenomics for part mining to find libraries of orthogonal repressors (73 synthetic genes) and transcription factors (62 synthetic genes). Thus, as the genetic networks and pathways of engineered systems in synthetic biology get larger and studies move to new organisms, there will be increasing reliance on de novo DNA synthesis to generate requisite system components.

493 An August 2013 statement by Iran on behalf of the Group of NAM and other States Parties to the BWC Meeting of Experts, reiterated in December 2014, highlighted 'due to the dual use nature of some of the new technologies, there is a potential for uses contrary to the provisions of the Convention including by programming cells to produce toxins, viruses or other cells which could cause harm, designing and building new or altered pathogenic viruses, the ability to confer mammalian transmissibility to viruses or drug resistance to pathogens, the decreasing genetic diversity and the development of incapacitating weapons and the increasing capacity to deliver biological weapons via the alimentary route'.

494 In May 2013, Amato *et al* reported the metabolic control mechanisms for persister formation in E. coli. Persister cells are one way that bacterial populations become resistant to antibiotics by responding to stress by signaling a small percentage of their population to enter into a dormant mode.

495 An April 2014 review of molecular mechanisms underlying bacterial persisters highlighted how recent technological advances in microfluidics and reporter genes have improved our understanding. The authors note that 'ubiquitous bacterial stress alarmone' is an emerging central regulator of multidrug tolerance and persistence and that 'in several different organisms, toxin-antitoxin modules function as effectors of… persistence'.

496 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'research papers devoted to creation of bacterial formulations were published in 2011-12 by scientists from Finland (lactic acid bacilli) and the USA (tularaemia, salmonellosis)'.

497 A July 2012 statement by Switzerland to the BWC Meeting of Experts noted that 'biological systems and even, to some extent, living organisms can be built from scratch and manipulated in ways that are unprecedented. Such sophisticated developments and the associated technology have enormous potential for public health, biomedical science, and agriculture. However, they may also pose risks to our societies as the same knowledge and the products and technologies involved can be misused to cause harm'.

498 In September 2011, Dymond *et al* reported completed the redesign and synthesis of a chromosome arm of yeast. This is the first step in an attempt to engineer an entire eukaryote genome to conform to three design principles: 'first, it should result in a (near) wild-type phenotype and fitness; second, it should lack destabilizing elements such as tRNA genes or transposons; and third, it should have genetic flexibility to facilitate future studies'.

499 In April 2014, Annaluru *et al* reported the successful redesign of the first full chromosome in the SC2.0 project. The authors demonstrated that the chromosome was full functional in Saccharomyces cerevisiae.

500 An October 2014 review of the convergence of biology and chemistry highlighted:

- The use of TALENs gene editing to 'add a function to a crop (herbicide-resistant cotton plant), remove functionality in a crop (non-pollinating corn to facilitate controlled hybridisation), and modify functionality (change pigment expression in flowers)';
- Using genomics, transcriptomics, proteomics and metabolomics approaches together 'to design organisms with desired properties. These tools have been applied in three different industrial projects: The production of lysine – a feed additive – using *Corynebacterium glutamicum*, production of xanthan – a thickener for use in food and personal care products – using *Xanthomonas campestris pv. campestris*, and production of acarbose – a medication used to treat type II diabetes – by *Actinoplanes sp.* For each of these projects it was critical to obtain information about the cellular machinery, from genome to metabolome, in order to rationally approach modifying the organism';
- Research to use CRISPR/CAS9 gene editing technologies to treat disease. The authors noted 'experiments on monkeys inactivating the genes for two human diseases, which poses the question of potential applications in humans';
- 'Using one-step synthesis, it is possible to generate nanoparticles coated with arrangements of hydrophobic and hydrophilic compounds on a length scale similar to biological materials'. The authors noted that 'these nanoparticles have been shown to have the ability to penetrate cell membranes. Such nanoparticles could either be designed to carry small payloads of highly active drugs (for example certain peptides), or the nanoparticle itself could act as a drug by interacting with viruses';
- 'A number of computational methods, software tools and programming languages have recently emerged in the life science community which have served to compress the laboratory time required for converting design of a recombinant vector to its delivery'.

501 A July 2012 working paper by China for the BWC Meeting of Experts highlighted 'an alternative cryptic approach to attack human population specifically through population specific microbes'. The authors also noted 'revelation of pathogenic microbes' genome evolution and its relation with infectivity and pathogenicity and greatly enhance the surveillance, diagnosis and therapy of related infectious diseases. There is no doubt that such DNA sequence information can also be used for the modification of antigenicity, infectivity, toxicity and drug resistance of traditional pathogens, even for the artificial design and synthesis of totally new pathogens, which will lead to the failure of traditional prevention and treatment of infectious diseases and make efficient prevention and control more difficult'. They also highlighted 'primary results of human microbiome researches... indicate that our normal physiological functions are closely related to our second genome, whose disorder might affect normal physiological metabolism of humans and even cause illness'.

502 In January 2011, Kim *et al* reported having developed preassembled zinc-finger arrays for rapid construction of Zinc Finger Nucleases, which are an important genome editing tool. The authors demonstrated using the array and having 'assembled and tested ZFNs to target a human gene and a mouse gene'. They noted that this technology enabled 'rapid construction of ZFNs in a few days using conventional recombinant DNA technology, and fewer ZFNs need to be synthesized to obtain a functional enzyme'.

503 A February 2011 review of optimized transcription activator-like effectors highlighted their potential for 'efficient genome editing and transcriptome modulation'.

504 In July 2011, Isaacs *et al* reported a mechanism for genome-wide codon replacement enabling the precise manipulation of chromosomes *in vivo*. The authors reported using two tools multiplex automated genome engineering (MAGE) and conjugative assembly genome engineering (CAGE) 'capable of fundamentally reengineering genomes from the nucleotide to the megabase scale'. They demonstrated having merged 'sets of codon modifications into genomes with 80 precise changes'.

505 In November 2012, Bedell *et al* reported using designed transcription activator-like effector nucleases (TALENs) for 'targeted zebrafish genome editing and functional genomic applications'. The authors demonstrated having 'used single-stranded DNA oligonucleotides to precisely modify sequences at predefined locations in the zebrafish genome through homology-directed repair'. They also showed successful germline transmission of engineered chromosomes.

506 An August 2014 statement by Switzerland to the BWC Meeting of Experts highlighted the use of CRISPR/CAS9 systems and that 'recent advances have now demonstrated that this feature of the bacterial immune system can be exploited to edit, silence and activate genes at any given site in virtually any kind of genome, including human cells. It promises to become a very powerful genomic engineering tool that may be very useful in terms of e.g. gene therapy or research'.

507 In July 2015, Schumann *et al* reported a significant step towards the clinical application of CRISPR/CAS9 genome editing. The authors reported having developed 'a programmable tool to replace specific nucleotide sequences in the genome of mature immune cells'. They noted their approach could 'efficiently "knock out" genes and "knock in" targeted genome modifications to modulate T-cell function'.

508 An August 2014 commentary on reports of CRISPR/CAS9 gene drives highlighted that inheritable selfish genes 'could also be used to alter populations of agricultural plants or livestock by actors intent on doing harm'. The authors noted 'gene drives and most other advanced applications of genomic engineering do not use proscribed agents or create regulated toxins and hence fall beyond the scope of operational regulations and agreements'.

509 An August 2014 presentation to the BWC Meeting of Experts highlighted the potential to use CRISPR/CAS9 gene drives to influence the inheritance of genes in sexually reproducing animals and plants with short reproduction cycles. The author noted implications for: 'gain-of-function enabling ability to host diseases; suppression of crops and livestock in traditional agriculture; suppression of pollinators and other keystone species Immunization drives may protect self and allies from effects; and the reversal drives may be withheld for economic or political gain.

510 A September 2015 review of the history of optogenetics, examines technical developments in the field over the last 10 years. The authors describe an exponential growth in papers being published with keywords associated with optogenetics since the Seventh Review Conference.

511 A November 2014 article in Nature highlighted the application of novel microscopy "technique that makes mouse brains transparent shows how the entire brain responds to cocaine addiction and fear". The authors note that such research "could uncover new brain circuits involved in drug response".

512 A July 2012 statement by Poland to the BWC Meeting of Experts highlighted 'the potential military, terrorist uses of molecules such as biological regulators and their dual use implications. In particular important features and military advantages of the new bioregulators are novel sides of toxic action, rapid and specific effect, penetration of protective filters and equipment and military effective incapacitation'.

513 A July 2012 working paper by the UK for the BWC Meeting of Experts highlighted 'potential military and law enforcement applications arising from... key advances in neuroscience, including neuropharmacology, functional neuroimaging and neural interface systems'.

514 A June 2014 review of the implications of the convergence of biology and chemistry highlighted:

- 'Drug companies have screened large numbers of metabolically resistant analogues [of peptides], mostly with unnatural (chemical) modifications, some of which may have substantially increased potency and toxicity. However, such modifications may add to the complexity and cost of the end product'
- 'Some peptides that cause bronchoconstriction, e.g. substance P, have been reported to have moderate to high inhalation toxicity in small rodent species. Such peptides interact with receptors on the surface of the lung which does not require penetration of membranes';
- 'Other types of bioregulators should also be noted. For example, some of those derived from lipid pathways, such as eicosanoids (prostaglandins, thromboxanes, leukotrienes) and platelet activating factor, have high physiological activity, and in some cases high toxicity (particularly metabolically less labile analogues). These have mostly been synthesised through multistage chemical processes, although biologically mediated stages are known'.

515 A July 2012 presentation by the Netherlands to the BWC Meeting of Experts highlighted that synthetic biology would facilitate using genes to programme cells to become 'microbial factories' to make a high value product, like pharmaceuticals, bioplastics, or biofuels, alternatively it could be used to make 'dedicated cells or viruses for misuse'.

516 An August 2012 review of synthetic biology highlighted progress in: DNA synthesis and assembly; design and production of biological parts; protein engineering; understanding, manipulating and building networks and pathways; synthetic life; software and modeling; and instruments.

517 A January 2014 review of synthetic biology in mammalian cells highlighted that 'a new generation of synthetic biology research tools' and 'programmable DNA-binding domains and RNA regulators are leading to unprecedented control of gene expression and elucidation of gene function. Rebuilding complex biological circuits such as T cell receptor signaling in isolation from their natural context has deepened our understanding of network motifs and signaling pathways'.

518 An August 2014 presentation by the OPCW to the BWC Meeting of Experts highlighted enabling technologies having resulted in 'an expanded capability to redesign or manipulate organisms' and 'to design and engineer improved enzymes'. The authors noted 'peptides are the largest group of bioregulators and have been the class for which commentators on the CWC have expressed most concern for misuse... Peptides could be produced using metabolic engineering and synthetic biology but the pharmaceutical industry currently regards multi-step chemical synthesis, using specialized equipment, as the most cost- effective method for producing many small peptides'. The authors noted that 'there are shortcomings of peptides as drugs'. An October 2014 review of the impacts of convergence of chemistry and biology highlighted that 'using tools that are already available, including direct insertion of gene sequences and directed evolution, organisms and molecules can be guided to meet a specific purpose within a reasonable timeframe and with a degree of reliability that was not previously possible. A number of examples were presented and discussed for industrial-scale production of complex molecules with application in medicine and elsewhere'.

519 A May 2013 review highlighted progress in combining computational design of enzymes with directed evolution, molecular dynamics simulations, and crowd-sourced structure-prediction approaches to result in significant improvements in binding, turnover, and thermal stability.

520 In November 2013, Cherny *et al* reported engineering enzymes to detoxify V-type nerve agents using computationally focused libraries. The authors used 'an integrated computational and experimental approach to increase Brevundimonas diminuta phosphotriesterase's (PTE) detoxification rate of V-agents by 5000-fold'. They experimental tested the results and fed them back to improve the computational models. After five rounds, the authors had variants that 'hydrolyze the toxic SP isomers of all three V-agents with kcat/KM values of up to 5 × 10(6) M(-1) min(-1) and also efficiently detoxify G-agents'.

521 A January 2015 review of tools for creating custom organisms in yeast highlighted 'computational tools for the prediction of biochemical pathways, molecular biology methods for assembly of DNA parts into pathways, and for introducing the pathways into the host, and finally approaches for optimizing performance of the introduced pathways'.

522 In September 2013, Procko *et al* reported the computational design of a protein-based enzyme inhibitor. The authors used computational design techniques to design a protein that binds to the acidic active site of hen egg lysozyme and inhibits the enzyme. The authors demonstrated experimentally that the designed protein 'bound lysozyme with low nanomolar affinity, and a combination of NMR studies, crystallography, and knockout mutagenesis confirmed the designed binding surface and orientation'.

523 An October 2014 review of the convergence of biology and chemistry highlighted:

- 'The technical capability for chemical synthesis of toxins exists, and synthetic biology approaches could also be used for toxin production and modification'. The authors noted recent research with Saxitoxins;
- The use of genome editing technologies to remove Ricin from the castor plant. The authors noted 'there were questions about whether it would be possible to use these techniques to increase (rather than remove) the number of copies of the gene sequence for one of the isoforms in the castor plant, to increase the concentration of Ricin toxin. Though this was deemed technically feasible, it remains questionable whether and why someone wishing to acquire large quantities of Ricin would not simply follow the low-cost and low-tech avenue of planting more castor plants'.

524 A May 2014 review of large-scale *de novo* DNA synthesis highlighted significant progress in efforts 'to engineer new protein functions by taking advantage of computational design and metagenomic information'. The authors noted 'the development of deep mutational scanning techniques to measure structure-function relationships in multiplex will enable rapid characterization of large designed synthetic gene libraries as synthesis methods improve'.

525 A March 2012 review of developments in vaccine production highlighted the use of suspended cultures which reduces the physical space required for vaccine production and significantly reducing cost by enabling batch processing of verification steps, and dramatically increasing surge production capacity. The authors noted that the 'actual facility and the equipment is much smaller for getting the same amount of doses'.

526 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted microreactors 'alter the signature of chemical production infrastructure to some extent, although they are only one piece of a sequence of infrastructure'. The authors noted they 'could shorten the time from the discovery of a new class of toxic chemicals to production of selected agents. There is also the possibility that other toxic chemicals (e.g., pharmaceuticals and peptides) could be produced in microreactors for prohibited purposes'.

527 An October 2014 review of the convergence of biology and chemistry highlighted:

- 'The potential of 3D printing for building customised reaction vessels, such as micro-reactors and the related processing equipment, which although limited in volume sizes… offers a means to subvert traditional controls on process equipment made of high performance and corrosion resistant materials'. The authors also noted '3D-printed tissue culture systems… potentially offered actors the possibility of lower production costs and materials used and, significantly, a smaller footprint for production';
- 'A number of computational methods, software tools and programming languages have recently emerged in the life science community which have served to compress the laboratory time required for converting design of a recombinant vector to its delivery'.

528 In February 2015, Kelwick *et al* reported having used a forward design approache to engineer E. coli to produce increasing amounts of a plastic. Having compiled a device for the production of the plastic the authors then developed a series of different devices using different promoters and ribosome binding sites. They reported a combination that resulted in a six-fold increase in plastic production. The authors also demonstrated thefeasibility of using non-recyclable waste as a low-cost carbon source for this biodegradable plastic.

529 An October 2014 review of the impacts of convergence of chemistry and biology highlighted that:

- 'Using tools that are already available, including direct insertion of gene sequences and directed evolution, organisms and molecules can be guided to meet a specific purpose within a reasonable timeframe and with a degree of reliability that was not previously possible. A number of examples were presented and discussed for industrial-scale production of complex molecules with application in medicine and elsewhere';
- Progress in industrial biology is helping 'to develop effective, scalable and robust processes that convert a renewable feedstock such as sugar, using a microorganism such as genetically engineered yeast, to a desired chemical compound at industrial scale'. The authors noted an example where 'to reduce time and costs, the company automated and miniaturised as many repetitive R&D processes as possible… Though the infrastructure for automation was expensive to develop (~$200 M), the cost per sequence to develop has dropped exponentially with the implementation of this process, and the rate at which new sequences are developed has increased by magnitudes;
- 'The response of the organisms to the environment of a fermenter is not predictable, and any new organism must be carefully tested to prevent failures at the pilot plant scale. The timeline for development of a new product has been substantially reduced… However, the cost and time required to produce a new viable, scalable method are still high'.

530 A March 2012 review of developments in vaccine production highlighted directed evolution to remove reliance on animal serum, reducing cost, stabilising ingredient chains, and assisting in meeting purification standards.

531 A November 2013 review of emerging nanotechnology-based tools for biology and medicine, highlighted the potential to use nanoparticles 'to partition bulk solutions into smaller volumes that contain discrete numbers of molecules or cells'. The authors noted 'libraries of droplets encoding different analytes such as drug compounds, viruses, antibodies, or enzymes could be screened against cells or other reactants'. They provide one example where this 'technology was applied to the directed evolution of the enzyme horseradish peroxidase, which generated mutants with catalytic rates >10 times faster than the parent, with a 1000-fold increase in speed and a 1 million-fold reduction in cost'.

532 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted:

- 'The production of toxic chemicals, including toxins, through biological synthesis (an aspect of the convergence of chemistry and biology); encapsulation and delivery through nanotechnology; and flow microreactors, which enable types of chemical reactions under conditions that were not previously technically feasible';
- 'Microreactors have now entered the chemical production toolkit for certain kinds of operations. They alter the signature of chemical production infrastructure to some extent, although they are only one piece of a sequence of infrastructure'. The authors noted the 'major advantages of microreactor technology include the safe production of hazardous, corrosive chemicals, as well as certain classes of biologically active chemicals, which could not be otherwise done under batch conditions'. They also highlighted they 'could shorten the time from the discovery of a new class of toxic chemicals to production of selected agents. There is also the possibility that other toxic chemicals (e.g., pharmaceuticals and peptides) could be produced in microreactors for prohibited purposes'.

533 A June 2014 review of the implications of the convergence of biology and chemistry highlighted:

- 'A variety of microbes have been modified for commercial production. Metabolic engineering methods and techniques are becoming more mature and diverse chemicals can be produced on a commercial scale by engineered organisms';
- 'Engineered yeasts and bacteria are being used in process development plants and large scale production facilities around the world to produce ethanol as well as other products';
- 'Biocatalysts (enzymes), usually immobilised, are being increasingly applied in the industrial production of bulk chemicals and pharmaceuticals. To overcome the limitations of naturally occurring enzymes, directed evolution has become an important tool for improving activity, selectivity, solvent tolerance and general robustness';
- Reprogrammed microorganisms have been created 'to produce biofuels by manipulating metabolic and biosynthetic pathways, and the production of drugs that are difficult to synthesise or which otherwise would need to be extracted from scarce raw materials. Recent examples include the production of anthranilic acids such as the anti-allergic tranilast in modified yeast strains, and the production of terpenoid compounds which form the basis of perfumes and many drugs, including novel antimicrobial drugs';
- 'A survey of synthetic biology products published in 2012 provides additional insight into applications under development. The survey identified 68 products across seven sectors (including biofuels, chemicals, energy, food, materials, and medicine) being developed by companies in 10 countries';
- 'Substantial advances in production methods for peptides have been made in the last two decades. Although they could be produced in genetically modified organisms, the pharmaceutical industry currently regards chemical synthesis as the most cost-effective method for producing many small peptides'.

534 An October 2014 review of the convergence of biology and chemistry highlighted:

- Using directed evolution 'to take existing, non-optimal enzymes and tailor their properties to drive chemical reactions with high selectivity and yield'. The authors noted that 'from an industrial perspective, this technology has the potential to increase efficiency and reduce waste in existing or new processes… This has already been demonstrated as possible by Merck Company, which, in collaboration with Codexis, revised a process to incorporate an artificial enzyme instead of a man-made catalyst after only 11 rounds of directed evolution';
- 'Growing research interest in toxins for applications in medical treatment, life sciences research, pharmaceuticals, and agriculture shows that technological advances are changing the way in which toxins are being produced and used';
- The use of TALENs gene editing to 'add a function to a crop (herbicide-resistant cotton plant), remove functionality in a crop (non-pollinating corn to facilitate controlled hybridisation), and modify functionality (change pigment expression in flowers)';
- Using genomics, transcriptomics, proteomics and metabolomics approaches together 'to design organisms with desired properties. These tools have been applied in three different industrial projects: The production of lysine – a feed additive – using *Corynebacterium glutamicum*, production of xanthan – a thickener for use in food and personal care products – using *Xanthomonas campestris pv. campestris*, and production of acarbose – a medication used to treat type II diabetes – by Actinoplanes sp.';

- Increasing miniaturization and automation, combined with the use of standardised biological parts – for example 'a "toolbox" of sequences that have specific effects (gene sequence promoters, tags, terminators, etc.) were identified and catalogued to allow for easier sequence development'. The authors noted such an approach 'increases quality control, aids in records keeping and project tracking, and has increased the rate of sequence development significantly. This has also reduced the technical skill level required to perform the synthesis'. The authors also highlighted the potential to combine this approach with directed evolution.

535 A May 2015 review buy Liu and Stewart of plant synthetic biology examined enabling tools including those for designing components and their assembly and deployment. The authors provided examples of engineering synthetic sensors, metabolic pathways, and plastids.

536 A July 2012 statement by Poland to the BWC Meeting of Experts highlighted that 'advances in science and technology alter possible methods of production for molecules like toxins and regulators. Therefore, this biologically active compound increasingly can be produced in large amounts through chemical synthesis or biological process'.

537 A July announcement reported 'the launch of the first commercial surfactant derived from microalgae oil'. The authors noted the use of 'a highly controlled fermentation process to convert sugarcane into oils of the highest purity and performance'.

538 A June 2014 review of biopharming highlighted progress in 'flexible and rapid engineering methods, combined with benefits of high volume expression for protein isolation, or seed-based long-term storage'. The author noted that a number of products are already under development and that the FDA had already approved a 'recombinant plant drug to treat a disease'.

539 An October 2014 review of the convergence of biology and chemistry highlighted the use of TALENs gene editing to 'add a function to a crop (herbicide-resistant cotton plant), remove functionality in a crop (non-pollinating corn to facilitate controlled hybridisation), and modify functionality (change pigment expression in flowers)'.

540 A June 2014 review of novel start-ups highlighted the emergence of virtual biotechnology companies. The authors describe a small number of new companies founded by a number of individuals with an idea. In some cases, the companies have no employees or laboratory space but outsource all the work to consultants.

541 An April 2013 review of single use bioreactors highlighted that 'A number of bioprocess analysts estimate that the entire single-use market has been growing at about 15–20% per year. Single-use bioreactors (SUB), in particular, are rapidly increasing in popularity and seem destined to stay'.

542 An August 2012 industry survey of 300 biomanufacturers 'indicated a clear preference' for single-use bioreactors. At the clinical scale two-thirds of respondents indicated that they expected to implement batch-fed single use bioreactors (as opposed to just under a third who expected to use stainless steel batch–fed bioreactors). At the commercial scale, over half of respondents expected to still be using stainless steel (which is a significant reduction from the current market share).

543 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted 'single-use or disposable bioreactor systems have also progressed; these are easily installed, reduce costs, streamline validation, increase product consistency and reduce overall turnaround times. Simple rocker bags are suited to cell-culture for virus vaccine production as an alternative to traditional methods of growth in embryonated eggs. Single-use or disposable components also feature increasingly in downstream processing equipment'.

544 An October 2014 review of the impacts of convergence of chemistry and biology highlighted 'the potential of 3D printing for building customised reaction vessels, such as micro-reactors and the related processing equipment, which although limited in volume sizes (at the moment: 10 ml or less) could nevertheless eventually allow someone outside a chemistry laboratory or plant to print production equipment configured specifically for a particular end product. The technology thus offers a means to subvert traditional controls on process equipment made of high performance and corrosion resistant materials'.

545 In 2011, the US National Academies of Sciences published a report reviewing the threat posed by fungal diseases to human, animal and plant health. The report addresses fungi as pathogens, noting their ability to 'cause disease in healthy humans and animals' as well as to 'endure adverse environmental conditions and thrive outside their host'. The impact on plants was also stressed, noting that fungal plant pathogens had been responsible for the Irish Potato Famine, the Southern Corn Leaf Blight epidemic of the 1970s as well as Dutch Elm disease. Comparatively little attention has been paid to the potential use of fungal pathogens as a weapon, despite their comparative environmental stability.

546 In March 2013, Wessman *et al* reported four different formulae and approaches for freeze drying bacteria. The authors compared cell survivability in competing approaches and identified key properties whose control can significantly influence survival rates.

547 An August 2013 working paper submitted by the United Kingdom to the BWC Meeting of Experts highlighted:

- 'The 'creation of a synthetic vaccine for foot and mouth disease'. The authors noted 'steps to engineer enhanced stability characteristics into the construct mean that it would not require cold storage and that it would be cheaper to produce and distribute';
- 'Encapsulation of vaccines in silk matrices has been evaluated with the MMR vaccine, demonstrating that the matrices were capable of stabilising labile vaccines for more than six months over a range of tropical temperatures'.

548 An October 2012 review of advanced materials and processing for drug delivery highlighted 'innovation in material chemistry allows the generation of biodegradable, biocompatible, environment-responsive, and targeted delivery systems. Nanotechnology enables control over size, shape and multi-functionality of particulate drug delivery systems'.

549 A June 2014 review of the implications of the convergence of biology and chemistry highlighted:

- 'The shortcomings of peptides as drugs (and by implication for uses prohibited by the Convention) can be moderated in several ways. Formulations, particularly associated with liposomes or nanocarriers, are being explored to enhance penetration of the blood brain barrier, overcome host defences, and target specific organs';
- 'Nanoparticle-based formulations are being widely explored for enhanced or 'smart' drug delivery. Examples are controlled drug release, enhanced penetration of the blood brain barrier (e.g. for therapeutic peptides), and targeting specific organs or cells (e.g. cancer cells). Nanoparticles most commonly used in drug formulations include: imprinted polymers, dendrimers, vesicles, nanospheres, nano-capsules, micelles, carbon nano-tubes, liposomes, and nano-emulsions. Additional bio-based nanocarriers are being researched including DNA-based systems and viral-based systems';
- 'Nanocarrier-based delivery systems present several advantages over the classic ones: overcoming solubility problems, protecting the drug from the external environment (temperature, UV radiations, pH), and controlling the release profile';
- 'Nanocarrier-based delivery systems permit a more precise and controlled targeting at the site of action, while reducing the time of exposure at non-targeted tissues. This can increase efficacy, and reduce toxicity and side effects'.

550 An October 2014 review of the convergence of biology and chemistry highlighted 'using one-step synthesis, it is possible to generate nanoparticles coated with arrangements of hydrophobic and hydrophilic compounds on a length scale similar to biological materials'. The authors noted that 'these nanoparticles have been shown to have the ability to penetrate cell membranes. Such nanoparticles could either be designed to carry small payloads of highly active drugs (for ex- ample certain peptides), or the nanoparticle itself could act as a drug by interacting with viruses'.

551 A February 2013 review of the impact of scientific developments on the Chemical Weapons Convention highlighted:

- 'The production of toxic chemicals, including toxins, through biological synthesis (an aspect of the convergence of chemistry and biology); encapsulation and delivery through nanotechnology; and flow microreactors, which enable types of chemical reactions under conditions that were not previously technically feasible';
- 'The application of nanotechnology to improve materials and systems relevant for protection against CW and chemical decontamination is an area of gradual, but important development. Some areas of ongoing research that should continue to be monitored include the development of nanofibers for protective clothing, nanocatalysts for decontamination methods, and nano-based drug delivery mechanisms for medical countermeasures'; and
- 'New polymers, lipids, and other carrier materials for drugs or genes is an area of active research. These carriers may be used as components of nanomedicines, in which the properties of the carrier influence the biodistribution of the therapeutic agent that is bound or complexed with it. Both the chemical properties of the carrier (e.g., the relative hydrophilicity and hydrophobicity or the conjugation of "functionalizing" molecules to target particular cellular receptors) as well as physical properties (such as size and shape) influence the uptake and residence time of a drug in the body. These types of nanomedicines have a wide range of applications, including improved delivery across biological barriers such as the blood–brain barrier, prolonged residence time in the blood (reduced clearance in the liver), and improved targeting to particular cell types'.

552 In January 2014, Kembel *et al* reported that architectural design drives the biogeography of indoor bacterial communities. Their analysis might be of use in modeling how an attack might be optimized in a closed space.

553 In September 2012, Emaneul *et al* reported a novel mechanism for tagging agents to study their environmental spread. The authors noted that 'the use of genetically tagged spores overcomes the ambiguity of discerning the test material from pre-existing environmental microflora or from previously released background material'. They demonstrated under field conditions that it was possible to detect the released agent and differentiate it from other tagged agents and wild-type spores. As a proof of principle, in September 2012, Buckley *et al* reported using this barcoding system to track a release of a B. anthracis simulant.

554 A May 2012 review of the use of nanopartices in drug delivery highlighted that 'nanoparticle suspensions which contain medicines i.e. 'nanomedicines') has made it possible to increase the therapeutic index of many components (improvement of the activity, reduction of toxicity) by selectively directing them towards the diseased tissues and cells ('drug targeting')'.

555 A June 2014 review of the implications of the convergence of biology and chemistry highlighted:

- 'The shortcomings of peptides as drugs (and by implication for uses prohibited by the Convention) can be moderated in several ways. Formulations, particularly associated with liposomes or nanocarriers, are being explored to enhance penetration of the blood brain barrier, overcome host defences, and target specific organs';
- 'Nanocarrier-based delivery systems permit a more precise and controlled targeting at the site of action, while reducing the time of exposure at non-targeted tissues. This can increase efficacy, and reduce toxicity and side effects'.

556 An October 2014 review of the impacts of convergence of chemistry and biology highlighted:

- 'Antibody-Drug Conjugates (ADCs) have been developed and tested for the targeted delivery of cytotoxic payloads to cancer cells… an approach has been developed which combines selectivity with toxicity, by linking antibodies and cytotoxic molecules together in the form of ADCs'. The authors note that 'at this stage of development, ADCs are expensive drugs, but the approach is considered competitive based on their increased efficacy. Moreover, two such approaches have met with FDA approval and some 30 other ADCs are currently in clinical trials at different phases. The Swiss company Lonza, for example, has developed both the capacity to produce bulk quantities and the specialist skills and experience in both the chemical and biotech fields necessary to manufacture ADCs. In terms of capacity, reactor sizes can vary from small (6 to 60 litres) to large scale (up to 600 litres, batch sizes up to 3 kg)';
- 'nanoparticles, specifically… gold particles coated with hydrophobic and hydrophilic compounds could be used to imitate certain biological functions…. these nanoparticles have been shown to have the ability to penetrate cell membranes. Such nanoparticles could either be designed to carry small payloads of highly active drugs (for example certain peptides), or the nanoparticle itself could act as a drug by interacting with viruses'.

557 In February 2012, Ellis *et al* reported having identified a more efficient way to deliver protein-based drugs into cells. The authors demonstrated using boronic acid to boost the uptake of a protein by over four times.

558 There have been a number of recent cases of individuals using the internet, dark web and other information technology platforms to acquire toxins. For example, in November 2014, the UK used its Biological Weapons Act for the first time to convict a woman of attempting to acquire Abrin to kill her mother. A second conviction followed in April 2015, when a 16 year old boy attempted to acquire Abrin as part of a suicide attempt. A third conviction followed in July 2015 when a man was convicted of attempting to acquire Ricin for unspecified purposes.

559 In March 2015, Zilinksis and Mauger reported having surveyed materials provided by online venders of technologies controlled under the Australia Group. The authors reported "that a significant proportion of them were particularly vulnerable to proliferator exploitation. These typically were small firms with limited resources, facing significant competition, and offering transactions of single units". The authors also noted that "online vendors routinely offered payment options that could be utilized by unscrupulous buyers to hide their identities and to launder funds". The also concluded "that a host of new biotechnology innovations had emerged in the last ten or more years that potentially have BW-related applications and are being offered for sale by vendors on the Internet".

560 A July 2012 working paper by the Russian Federation for the BWC Meeting of Experts highlighted 'Current methods in molecular biology [which] provide means to modify pathogens the way that will make it difficult to identify them by existing means of identification'. The author noted 'this hampers significantly the effectiveness of diagnosis and preventive vaccination'.

561 An October 2014 review of the convergence of biology and chemistry highlighted 'the potential of 3D printing for building customised reaction vessels, such as micro-reactors and the related processing equipment, which although limited in volume sizes… offers a means to subvert traditional controls on process equipment made of high performance and corrosion resistant materials'. The authors also noted '3D-printed tissue culture systems… potentially offered actors the possibility of lower production costs and materials used and, significantly, a smaller footprint for production'.