Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

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Standing agenda item: review of developments in the field of science and technology related to the Convention

Developments in science and technology - diagnostics

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I. Background

1. The Seventh Review Conference called for "…review of developments in the field of science and technology related to the Convention."[1] The goals of this agenda item are to understand the implications of new science and technology and their relevance to how the convention is implemented. Discussions under this item should increase shared understanding of the implications of new developments in the life sciences, including risks and benefits, to assist States Parties in making informed choices about how to manage risks.

2. The specific topics to be addressed at the Meeting of Experts in 2013 are "advances in technologies for surveillance, detection, diagnosis and mitigation of infectious diseases, and similar occurrences caused by toxins in humans, animals and plants..."1 This paper surveys some recent and emerging technologies in the field of infectious disease diagnostics as they relate to surveillance, detection and diagnosis.

II. Summary

3. Diagnosis is the process of identifying the cause of an individual's symptoms (or, less frequently, of detecting the presence of a disease agent in an asymptomatic individual). The timely and accurate diagnosis of infectious disease is crucial for determining appropriate treatments, for identifying the origin and following the spread of disease-causing microorganisms (pathogens) in populations, and for developing strategies to limit

[1] BWC/CONF.VII/7.

spread. From a public health standpoint, a shorter time between outbreak detection and diagnosis permits the more rapid application of mitigation and containment measures, which leads to fewer people being infected.

4. As a practical matter, most disease diagnosis is performed at the point of care (POC) by the recognition of characteristic symptoms, rather than by the use of tests that confirm the presence or absence of specific pathogens. If available, standard microbiological isolation and characterization can provide confidence that the exact cause of illness has been identified, although such procedures can be slow. For some infectious diseases, generally inexpensive POC devices are available that can increase the speed and accuracy of diagnosis. 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 both at POC and at centralized laboratories by providing more rapid identification and more precise information about known and, in some cases, newly emerged pathogens.

5. A trend in recent years is to make sophisticated tests, or assays, more easily performed with less training, leading to decentralization and diagnosis closer to the POC.[2] A countervailing trend is 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.

6. Although many types of technology have been applied to diagnostic testing, a majority of diagnostics tests belong to one or both of two major classes - immunoassays and molecular assays. For immunoassays, advances in technology have led to an increased speed of diagnostic test development using nucleic acid[3] sequence information and synthetic biology for manufacturing specific antigens or antibodies used in those tests. 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.[4, 5] 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. The implications of some of these “enabling technologies” have been discussed in previous BTWC working papers.[6] In addition to these general approaches, many others that have been proposed or are being developed; some are mentioned here in the context of emerging technologies.

III. Advances in diagnostics

1. Immunoassays

7. Antibodies are the key to immunoassays; they are proteins that bind targets, called antigens, with high specificity as part of the normal immune response. Diagnostic tests take advantage of this specificity. For example, antibodies in blood or saliva can bind to specific

[2] Mbanya, D. 2013. Clin. Microbiol. Inf. 19:416.
[3] Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are nucleic acids that comprise genetic information and are targets for many diagnostic tests.
[4] Curtis et al. 2012. PLoS ONE 7(2): e31432.
[5] http://sites.path.org/dx/hiv-stis/nina/.
[6] BWC/MSP/2012/MX/WP.6.

antigens attached to a plastic support or nanoparticle, thereby indicating an individual's exposure to a particular pathogen. Alternatively, attached antibodies can be used to capture pathogen antigens from a sample.

8. Advances in DNA sequencing and synthetic biology have enabled development of immunoassay diagnostics based on microbial genome sequences.[7] For example, in the case of MERS-CoV, a coronavirus causing outbreaks in the Middle East and Europe, sequencing of the viral genome enabled rapid development of molecular tests and immunoassays. The immunoassay uses a viral antigen, generated by synthetic biology technology.[8] Such rapid assay development illustrates the beneficial conjunction of multiple technologies enabling studies of population exposure, which in turn helps with outbreak mitigation and source identification.

9. There is a recognized need for rapid POC diagnostics in low-resource environments to provide near-real-time assessment of disease outbreaks.[9] Lateral flow immunoassays exemplify such devices; they use a simple test strip through which a sample (serum, saliva or urine) flows.[10] Perhaps the most familiar example of a lateral flow immunoassay is the modern home pregnancy test, which yields quick, easy-to-interpret results. Lateral flow immunoassays have been developed to detect many pathogens and toxins, including human immunodeficiency virus (HIV), hepatitis C virus, human papilloma virus, and malarial parasites,[11] and their utility at the POC continues to grow. Lateral flow immunoassays are generally inexpensive, accurate, have good shelf lives, and require little training to use, but they cannot identify unknown pathogens.

2. Nucleic acid-based diagnostics

10. Nucleic acid-based diagnostics rely on amplification and detection of DNA or RNA from a pathogen. As in immunoassays, only those organisms that are tested for are detected. PCR is the traditional method underlying molecular diagnostics, but isothermal amplification is gaining traction.[12] Both approaches yield billions of copies of a DNA or RNA sequence – detection of these copies constitutes the "signal" that a particular pathogen is present. Such signal amplification means that nucleic acid-based diagnostics are extremely sensitive and that near-real time quantification is possible as the signal accumulates. Also because of its sensitivity, nucleic acid amplification must be done with care to avoid cross contamination of samples. Because of their high specificity and relative ease of development, nucleic acid-based diagnostics can rapidly be developed as kits to detect virtually any organism.

11. Isothermal amplification of nucleic acids is increasingly being adopted for diagnostic testing; it does not require expensive equipment and is thus potentially useful in lower resource POC environments.[13] Several varieties of isothermal amplification have been developed.[14, 15] For example, Recombinase Polymerase Amplification has recently

[7] http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm.
[8] Reusken, C. et al. 2013. EuroSurveillance 18(14):pii=20441.
[9] http://www.dfa.org (Diagnostics For All is an example of a non-profit enterprise dedicated for POC diagnostics).
[10] Hanafiah, K.M. et al. 2013. Biomark Med. 7:333.
[11] Anfosi, L. et al., 2013. Anal. Bioanal. Chem. 405:467.
[12] Craw, P., and W. Balachandran. 2012. Lab Chip 12, 2469-2486.
[13] Niemz, A. et al. 2011. Trends Biotech. 29:240.
[14] Craw, P. & W. Balachandran. 2012. Lab Chip 12:2469.
[15] Several isothermal amplification methods have been developed: Transcription Mediated Amplification (TMA; Gen-Probe); Loop Mediated Isothermal Amplification (LAMP; Meridian);

been used to develop POC kits for diagnosing HIV infection,[16] methicillin resistant Staphylococcus aureus[17] and a variety of biothreat agents,[18] among others. The technology can detect fewer than ten copies of a pathogen's DNA or RNA with high specificity, in an hour or less at room temperature. The Genie II system (Pro-Lab Diagnostics; Pro-lab.com) is an example of a commercial open source platform for isothermal amplification assays, and other isothermal amplification system are being specifically developed for resource-limited settings.[19]

12. Nucleic acid-based diagnostics can be combined, or multiplexed, to create "panels" that simultaneously test for a variety of pathogens. Different commercially available multiplex nucleic acid-based diagnostics yield substantially similar results with similar costs.[20] The advantages of multiplexing are the ability to detect many pathogens in a single, rapid (1-7 hrs) assay and the potential to add panels as new pathogens emerge. An example of PCR multiplexing is the FilmArray system (BioFire Diagnostics; Biofiredx.com) that includes panels for dozens of pathogens. The xTAG system (Luminex; LuminexCorp.com) is an open source platform for designing custom diagnostic tests that can also be multiplexed.

13. Nanoparticles (gold, carbon, magnetic) can be adapted for rapid detection of pathogens by adhering DNA or proteins (e.g., antibodies or antigens) to their surface. An example of this approach is the Verigene system (Nanosphere; nanosphere.us), in which PCR products amplified from pathogen DNA capture specific DNA-gold particle complexes and generate a detectable signal. This Verigene system has been multiplexed to detect "panels" of organisms that commonly invade blood, lungs or intestinal tracts, along with genes involved in antimicrobial resistance.

3. DNA sequencing

14. DNA sequencing was discussed as an enabling technology in a 2012 MXP working paper.[21] Sequencing has become increasingly important for conducting surveillance, identifying origins of outbreaks, and diagnosing emerging pathogens.[22] Limited sequencing of a few genes encoded in pathogen DNA has become a common adjunct to standard laboratory microbiological testing and can identify pathogens at the strain level. High-throughput sequencing, which simultaneously yields millions of DNA sequences, has become easier, faster and less expensive, and can be used to discover unknown pathogens as well as their biological characteristics.

15. Several technologies are available for high-throughput sequencing. The MiSeq/HiSeq platforms (Illumina, Inc.; Illumina.com) yield millions of short DNA sequences per assay that can be assembled into longer segments until the entire genome is sequenced. With data stored and analyzed in the "cloud," the entire process can be monitored and assessed from a mobile device. The PacBio RS system (Pacific Biosciences; PacificBiosciences.com) sequences longer pieces of DNA and requires very little DNA to

Strand Displacement Amplification (SDA; Becton-Dickinson); Nucleic Acid Sequence-Based Amplification (NASBA; bioMerieux); Helicase Dependent Amplification (HDA; Biohelix) and Recombinase Polymerase Amplification (Alere; RPA).

16 Boyle, D. S. et al. 2013 mBio 4:e00135-13.

17 Piepenburg, O et al.. 2006. PLoS Biol. 4:e204.

18 Euler, M. et al. 2013. J. Clin. Microbiol. 51:1110.

19 http://www.path.org/publications/files/TS_update_nina_device.pdf.

20 Popowich, E. B. et al. 2013. J. Clin. Microbiol. 51:1528

21 BWC/MSP/2012/MX/WP.6.

22 Didelot, X. et al. 2012. Nat Rev Genet.13:601.

retrieve a nearly complete bacterial genome sequence in a single run.[23] Ion Torrent (Life Technologies; iontorrent.com) is a competing technology for whole genomes that uses hydrogen ions generated during sequencing as signals.[24] Nanopore-based sequencing (Oxford; nanoporetech.com), in development, aims to sequence whole genomes on a universal serial bus (USB) device using signals emitted as single DNA strands are ratcheted through a nanopore in a membrane.[25]

16. The preceding sequencing technologies can be used to study genomes, microbiomes and metagenomes. Microbiome refers to all microbes living in an environment, for example the "gut microbiome." Metagenome refers to all DNA sequences present in a sample (blood, tissue, feces, soil, etc.) including host, bacterial, viral or parasite DNA. Alterations in the species composition of the microbiome of the human gut have been linked to chronic disease states (e.g. Crohn's disease, obesity, cancer, diabetes) and can be used to monitor endemic diseases in a population.[26] The metagenome can be used for identifying unknown viruses or bacteria by subtracting human sequences and focusing on known or novel microbial sequences.[27]

17. High-throughput sequencing analyses are being applied to assess the overall state of health of an individual or population[28] for identifying unknown microorganisms and for providing a high degree of precision in tracing outbreaks and determining if an outbreak is natural, accidental or deliberate. For example, sequence-based epidemiology has been used for investigating outbreaks of methicillin resistant Staphylococcus aureus (MRSA)[29] and Klebsiella bacteria in hospital settings[30] and will likely become a standard tool for monitoring and determining the source of outbreaks.

4. Emerging and maturing technology

18. The scientific literature is replete with new ideas for diagnostics that may become useful for surveillance, detection, diagnosis and mitigation. The practicality of any one approach depends on many variables, including cost, benefits over current technologies, and sustainability. Mass spectrometry has been rapidly adopted for diagnostics in recent years because of its speed, sensitivity and accuracy, with several types of applications in development.[31, 32, 33] Other advances will likely come from work on microfluidic devices,[34, 35] paper-based fluidics,[36, 37] and combined fluidics and optoelectronics that interface devices such as microscopes or electronic readers with cell phones.[38, 39, 40] Other

[23] Chin, C. 2013. Nature Methods 10:563.
[24] Ramos, R.T. 2013. Microb Biotechnol. 6:150.
[25] Schneider, G. F. & C. Dekker. 2012. Nature Biotech. 30:326.
[26] Cho, I. & Blaser, M. L. 2012. Nature Rev. Genet. 13, 260.
[27] MacConaill, L, M. Meyerson. 2008. Nature Genetics 40:380.
[28] Yatsunenko, T. et al. Nature 486, 222–227.
[29] Kupferschmidt., K. 2012. Science 338:1019.
[30] Snitkin, E. S. 2012. Sci Transl Med . 4:148.
[31] Croxatto et al.. 2012. FEMS Microbiol. Rev. 36:380–407.
[32] Bizzini et al. 2010. J. Clin. Microbiol. 48:1549.
[33] Doellinger, J. et al. 2012. Mol. Cell. Probes 26:177–181.
[34] Mao, X. and T. J. Huang. 2012. Lab Chip 12:1412.
[35] Chin, C. D. et al. 2011. Nat. Med. 17:1015.
[36] Martinez, A. W. et al. 2008. Anal. Chem. 80:3699.
[37] http://www.genomeweb.com/clinical-genomics/uw-leads-10m-dod-funded-project-develop-paper-based-remote-mdx-device.
[38] Mudanyali, O. et al. 2012. Lab Chip, 2012,12, 2678.
[39] http://org.ee.ucla.edu/undergrad/.

types of detection technologies under development include surface plasmon resonance, which detects alterations in light when a target (e.g. antibody, antigen, cell) binds to a gold coated surface,[41] and electrochemical sensing, in which biosensors fabricated on microchips detect electrochemical signals from reactions taking place in solution or on a surface, for example when DNA binds to a probe.[42] These emerging approaches have the potential to be multiplexed to detect hundreds of targets.[43]

IV. Implications

19. The above discussion of developments in diagnostics suggests some general implications:

(a) New diagnostics are emerging from multidisciplinary collaborations that combine different approaches and understandings and distill inherently complex science and technology into simple devices that can be quickly deployed in the event of a disease outbreak. However, most advances in diagnostics still rely on the basic concepts outlined in this paper.

(b) The advances described herein strengthen and accelerate the ability of States Parties to detect and react quickly to an infectious disease outbreak, whether natural or deliberate, but have room for improved portability, sensitivity and specificity in dealing with unknown threats in complex samples.

(c) The depth and breadth of analysis enabled by new diagnostics should reduce misunderstandings about sources of disease outbreaks, while building awareness about the pace and tempo of disease emergence.

(d) Many new technologies are affordable for large reference or university-based diagnostic laboratories but are inaccessible to smaller units of health care. Thus, there is a need for developing inexpensive and mid-range technologies that can make scientific advances more readily available.

(e) To be effective in disease surveillance, detection, diagnosis and mitigation, new and emerging technologies must include both POC devices for surveillance and platforms with higher resolution to deal with the problems of emerging diseases and acute outbreaks.

(f) Advances in these areas create a safer and more secure world because of an increasing ability to detect, diagnose and mitigate emerging diseases, and because of the greater precision in determining the source and likely progression of infectious microorganisms.

(g) To the extent that molecular and immunoassay tests are available, affordable and effective, they can also reduce reliance on culturing pathogens and on collections of reference samples and thus offer potential biosafety and biosecurity benefits.

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[40] Gallegos, D. et al. 2013. Lab Chip 13:2124.
[41] Marusov, G. et al. 2012. Environ. Sci. Technol., 46:348.
[42] Lam, B. et al. 2012. Anal Chem. 84:21.
[43] Lam, B. et al. 2013. Nat. Comm. Doi: 10.1038/nccomms3001.