# 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

7 August 2013

English only

**2013 Meeting**
Geneva, 9–13 December 2013

**Meeting of Experts**
**Geneva, 12–16 August 2013**
Item 6 of the provisional agenda
**Standing agenda item: review of developments in the field of science and technology related to the Convention**

## Advances in laboratory diagnostics, point of care detection, pathogen characterisation and potential benefits to the Biological and Toxin Weapons Convention

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*Summary*

Technology is advancing at a rapid pace in many divergent field of science. These advances may be applied to the detection and characterisation of pathogens which in turn aids patient care. Faster and cheaper methods of disease detection and characterisation benefit not only the patient but also have defensive applications. Techniques such as Polymerase Chain Reaction (PCR), immunochemistry, electro-chemical detection and mass spectrometry are currently used by first responders and military personnel to detect potential threat agents. Therefore, any advance made in either of these fields has the potential to aid against bioterrorism and biowarfare in that incidents may be dealt with faster and more accurately. Populations may be monitored for outbreaks of known and unknown agents using techniques such as Next Generation Sequencing. This lowers the appeal of using these agents as means of warfare or terrorism, thereby strengthening the Biological and Toxin Weapons Convention.

## I. Introduction

1. Various advances have been made in the fields of molecular biology, nanotechnology, microfluidics and chemistry that can potentially aid in the rapid diagnosis of disease. This would ultimately benefit the patient through initiating therapy faster as well as monitoring therapeutic efficiency in a more cost effective manner.

2. More accurate and cost effective methods of pathogen characterization aid epidemiological studies and serve to further the promotion of public health and disease control. Next Generation Sequencing (NGS) technology has made large scale DNA sequencing more affordable which in turn leads to greater depth in phylogenetic studies. Additionally, the concept of RNA-seq, in which gene expression studies are carried out on NGS platforms, offer a viable alternative to microarray-based experiments.

3. These potential breakthroughs in public health may also have defensive spin-offs. Techniques used to rapidly detect and characterise common human pathogens, may also be utilised for biological warfare agents. This would be advantageous not only in expediting incident management but also for epidemiological (forensic) studies in securing the maximum amount of information regarding a biological weapons incident in the shortest amount of time.

4. A review such as this serves only to touch on aspects of technological developments relevant to the Convention, with each subsection finding extensive scope for review in its own right.

# II. Advances in pathogen detection

5. The rapid detection of pathogens in the laboratory and in the field is of paramount importance. From a clinical standpoint, if diseases are diagnosed quickly and cost effectively patient treatment can be initiated faster. Also, by identifying the causative agent a more tailored therapeutic approach may be utilised, which in the end leads to a better clinical outcome. Technology developed for medical diagnostics may easily be used for environmental analysis in the field of bio-defence. Besides utilising a reliable laboratory-based identification and characterisation scheme, first responders often rely on field based detectors, which from a clinical perspective could be seen as “point of care” detection.

6. One of the primary aims of diagnostic technology development is to reduce turnaround times. This may often be achieved by developing diagnostic methods that are used at the “point of care” i.e. near the patient. This means that the technology should be user friendly enough for a non-laboratory worker (e.g. nurse or doctor) to use. In order to make this approach viable, assays should be comparable in cost, quality (sensitivity and specificity) and turnaround time to laboratory-based techniques. Often times, point of care testing is limited to screening only and not highly sensitive diagnostics.

7. Tuberculosis (TB) may be seen as the model disease that necessitates point of care diagnostics. Often times TB is misdiagnosed or confirmatory laboratory diagnosis is made weeks after a patient presents to a healthcare worker. This greatly hinders effective and rapid treatment initiation which can in-turn lead to the development and promotion of the disease within the community. 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.[1]

8. Mass/Piezoelectric detectors make use of quartz crystals that are highly sensitive to changes in the physical characteristics of a molecule. The technology may monitor the interaction between a target and a ligand or a particular chemical reaction. As an example the “Quartz Crystal Microbalance” which may be utilised for the detection of TB relies on monitoring the gravitational load and viscosity properties of a sample in order to generate a frequency shift in a quartz crystal resonator. The detector’s active surface is coated with TB antibodies which capture TB cells, leading to a frequency shift in the crystal. This may be detected in realtime as a sample is introduced.[1][2]

9. From a simpler, lower technology standpoint, the classical ELISA (Enzyme Linked Immunosorbancy Assay) may be made field portable using lateral flow chromatography. This technology, commonly known for its use in home pregnancy testing, may also be used for, amongst others, tuberculosis screening in the field especially, in resource limited settings.[3][4] In addition to rapid patient screening, the technique may also give clinicians an indication of the target organism's susceptibility to treatment. Ochang and colleagues[5] demonstrated the efficacy of identifying isoniazid and rifampicin resistant organisms from broth cultures using these strips. The technology has been used widely by first responders in biological terrorism field-work and research continues to improve sensitivity for the detection of threat agents such as botulinum.[6]

10. Polymerase Chain Reaction, originally developed in the early 80's is rapidly moving from the laboratory to the patient's bedside. As an example, Idaho Technologies has developed an array-based realtime PCR system that combines sample preparation, PCR amplification and data analysis onto a single instrument with very little user involvement. When comparing the Idaho Technology Inc. FilmArray to traditional methods and the FDA approved xTAG RVP (Luminex) using retrospective patient samples for respiratory viruses, Rand and Co-workers[7] found that the FilmArray outperformed both traditional methods and the xTAG RVP assay. The FilmArray assay is carried out by adding distilled water and a patient sample to two separate ports on a sample analysis pouch respectively using standard syringes. The advantage of this simple technology for First Responders in a Bioweapons/Bioterrorism incident is evident.

11. Researchers have modified PCR chemistry in such a way as to simplify the instrumentation required to perform these assays. The heating and cooling required for PCR requires precision instrumentation that is energy and cost intensive, and therefore not ideal for resource limited settings. An example of an alternative is loop mediated isothermal amplification (LAMP) technology.Lui and colleagues integrated DNA extraction and amplification for the detection of TB using a magnetic bead LAMP-based microfluidic system. The sample or reagent is introduced sequentially into a polyterafluoroethylene capillary and made to form droplets, in which the reactions take place. Amplification products are detected by fluorescence and 10 samples may be run simultaneously, yielding results within 50 minutes. The assay was found to have a lower limit of detection of 10 cfu.[8]

12. Huang and colleagues have taken the isothermal paradigm a step further, and developed a system that does not require electricity (socket or battery) to perform *Clostridium difficile* diagnostics in extremely resource limited settings. 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.[9]

# III. Advances in pathogen characterisation: NGS

13. Over the past few years there has been a marked shift from automated Sanger sequencing (e.g. capillary electrophoresis) to Next Generation Sequencing (NGS). Sanger methods have yielded unprecedented milestones in biology, such as the first validated human genome sequence,[10] and is by no means outdated. Yet, the depth and reduced cost provided by NGS makes it extremely attractive even though the technology is in its infancy.

The vast quantity of data generated by this technology is its trump card as well as one of its major pitfalls.

14. Since its inception in 2005 NGS has utterly shifted the paradigm in genomics research.[11] Projects that were unaffordable before are now entirely possible using this technology or by subcontracting projects to any one of a number of commercial NGS laboratories. NGS technology as it stands today consists of three main manufacturers: Lifetech (e.g. SOLiD, Ion Torrent and the Proton), Illumina (e.g. HiSeq 2000 and MiSeq) and Roche (e.g. GS FLX Titanium). [12] Each instrument functions on a different chemical principle and produce varying data volumes and depths at widely divergent costs.

15. Currently, NGS platforms utilize a three step analytical process. Firstly, library preparation, in which DNA molecules are sheared or synthetically (PCR or cloning) shortened to the appropriate length.The enrichment step serves to physically separate DNA molecules by labeling them with specific primers. The third step, analysis, is platform dependent and relies on either pyrophosphate release (Roche GS FLX Titanium), fluorescent detection (e.g. IlluminaMiSeq) or hydrogen ion release (e.g. Lifetech Ion Torrent). [13]

16. The Lifetech Personal Genome Machine (PGM) requires a special mention as its technology type is quite different from its competitors as it relies on semiconductor sequencing technology as opposed to light based detection. When a nucleotide is incorporated into a growing DNA chain a hydrogen atom is released.[14] The slight change in pH induced by this release is measured by the instrument. The voltage change induced by the nucleotide (after sequential flooding of the chip) allows the instrument to characterize which nucleotide is added to the growing chain. If two of the same nucleotide is added then the voltage would double. This is the first instrument that doesn't rely on fluorescence or cameras to process data; thereby reducing cost and instrument footprint.[12]

## A. Metagenomics and next gen sequencing

17. One of the most rapidly expanding fields in microbiology is metagenomics[13], which is a sub-discipline that describes the analysis of the total nucleic acid content of a particular sample. This approach allows researchers to characterise a microbial community without purification and subculture of individual community members.[15]Evolutionarily conserved marker genes in bacteria, fungi and archaea are used to identify the members of a mixed population. A problem that has hindered microbiology since its inception as a field of study has been the difficulties associated with culturing microorganisms. To date only a fraction of known organisms can be isolated in pure culture.[16]Besides identifying organisms, through the in-depth characterisation of complex microbial communities we may now begin to draw inferences with regards to their interactions with the host organism (e.g. gut microbiomes)[17] or their immediate environment (e.g. soil microbiome).[15]

18. The clinical applications of these techniques are extremely diverse. As an example, the leading cause of morbidity and mortality in cyctic fibrosis (CF) patients is lung infections. Therefore, these patients stand to gain a great deal if clinicians are able to rapidly identify respiratory pathogens in a mixed sample. NGS 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. There are a number of caveats and drawbacks though. Data processing and interpretation requires a high level of skill and judgement on the part of the researcher. This level of bioinformatics expertise is not always available to the routine diagnostic laboratory. Additionally, the 16S rRNA gene does not always yield species level identification.[18] With that said though it is widely believed that this technology would find its way into the clinical diagnostic laboratory.

19. In addition to the plethora of bacterial species inhabiting the human body (commensally or as a result of infection) even more viral particles are present. These microbes lack commonality in their genetic structure and are often difficult or impossible to isolate in culture. The metagenomics approach allows researchers to characterise the so-called virome (total viral compliment of a host).[16] 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.[19][20]

## B. Biomarker studies

20. Traditional gene expression based biomarker studies are carried out using either fluorescent quantification of PCR products or micro arrays. The former being labour and cost intensive due to the low throughput and the latter being cost and time intensive due to the need to validate individual findings using PCR. The concept of RNA-seq, using NGS technology, has shifted this paradigm and it is widely accepted that NGS RNA-seq will outcompete microarray-based studies in the future. Furthermore, by sequencing the actual amplicons one not only gains copy number information (for relative gene expression studies) but also actual sequence data which could lead to the identification of post transcriptional modifications such as splice variants. This is not always possible with the probe-target paradigm of microarray technology. This means that the NGS experiment may be conducted without *a priori* knowledge of the transcript's sequence. In an RNA-seq experiment, mRNA is converted to cDNA which is used to prepare the sequencing library. An NGS platform will then sequence the library and the relative intensity of each fragment may be used to determine comparative gene expression data.[21]

21. It has been found that a number of diseases are associated with expression changes in a specific set of disease-specific genes. As an example the OncotypeDx assay probes the expression of a panel of seven biomarkers and five reference genes in order to predict the recurrence of cancer in a patient.[22] It could be easily predicted that 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.

22. This concept extends to biomarkers of exposure to specific pathogens. As an example, bovine tuberculosis remains a problem in a number of nations and serodiagnostic techniques have failed to accurately diagnose the disease. Genomic markers of exposure promise to provide biomarkers in non-symptomatic animals which would aid in the eradication of the disease. Researchers are currently studying the host-pathogen interaction *in vitro* using NGS technology, in hope of identifying accurate markers of disease.[23]

23. It is important to understand the interaction between the host and a pathogen in order to implement effective and novel treatment strategies as well as early diagnostics. With the advent of RNA-seq technology one is now able to simultaneously determine the pathogen as well as the host's gene expression profile during infection.[24] This will hopefully lead, in the near future, to the diagnosis of disease prior to the onset of symptoms.

# IV. Advances in toxin detection

24. Besides utilising immunological methods for the detection of toxins, the field of mass spectrometry (MS) has a lot to offer when considering protein identification and sequencing. MS broadly describes the ionisation and subsequent measuring of an analyte's (or its fragments) mass; thereby generating a fingerprint that would aid in identification.[25] The drawback however is that the machinery required for these analyses is often expensive

and not field portable. This paradigm is however rapidly changing with the miniaturisation of various MS instruments and the development of field-amenable MS sources (sample introduction and ionisation interfaces).

25. Smaller molecules are readily made volatile and analysed by gas chromatography mass spectrometry (GC-MS) yet the same cannot be said for the vast majority of biological molecules (proteins and nucleic acids). With the advent of electrospray ionisation (ESI) and matrix assisted laser desorbtion/ionization (MALDI) ion sources, a great number of doors have been opened in the biological sciences using mass spectrometry. Methods of sample introduction (without pre-preparation) continue to evolve with the development of techniques such as Matrix Assisted Ionisation Vacuum (MAIV)[26] and Desorption Electrospray Ionisation (DESI)mass spectrometry.[27]

26. Of the wide variety of MS detectors available, 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 such as MALDI and ESI.[25] Field portable TOF systems have been investigated for defensive purposes over the past number of years[28] and may prove useful to first responders in the identification of biological as well as chemical warfare agents.

# V. Conclusions

27. The fields of molecular biology and chemistry are rapidly advancing to deliver novel tools that stand to benefit mankind. These technologies will not only yield a greater understanding of our natural environment but also fulfil much more basic needs, such as providing optimal healthcare to people in need. The first step in this road is the accurate diagnosis and characterisation of disease.

28. Many of the techniques discussed can, and have been, applied to the detection of biological warfare agents. The potential benefit to the Convention is obvious. The faster a first responder identifies a threat organism the faster remedial action can be taken and hence reducing the efficacy of a biological attack. Once these weapons become easier to detect and deal with, they become less attractive as agents of terror.

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