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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Investigations into Alleged Use of Biological Weapons – Some Considerations Drawn from Experience in the United Kingdom for Sample Handling and Biological Analysis

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Introduction

1. Experience within the UK, drawn from planning for sampling and analysis of suspected BW agents following use by hostile forces on the battlefield or following possible terrorist incidents, has highlighted a number of considerations relating to the handling of the samples, the facilities in which they are handled, and the techniques used to undertake analysis.

Reception and screening of samples

2. One of the first things to be noted is that some samples collected after an event may contain chemical or radiological agents in addition to, or instead of biological agents. Thus the methods for screening samples must be undertaken in such a way as to provide protection for the analysts handling unknown CBR materials in each of these eventualities. In addition, the screening methods must as far as is possible preserve the forensic integrity of the samples, just as the forensic audit trial must also be maintained at every stage from the sample's arrival, through the handling and analytical process. This means that the integrity of the chemical, biological or radiological agents must be preserved in the samples themselves, as well as other forensic evidence such as fingerprints and DNA.

3. The presence of infectious biological agents in a sample presents potentially the greatest immediate hazard for the persons undertaking screening after the sample is opened. Therefore, following initial radiological screening, unknown samples must be placed in adequate and appropriate microbiological containment before further screening procedures take place. Such

microbiological containment should also be designed to provide additional protection against chemical and radiological hazards.

4. In the UK, screening and analysis of suspected biological warfare agents is undertaken, at least initially, in a biological safety level (BSL-)3 high containment laboratory. If initial analysis indicates the presence of BSL-4 pathogens, such as Ebola or variola viruses, then the samples would be transferred to a BSL-4 maximum containment laboratory for further analysis and storage. The BSL-3 laboratory is equipped with fully enclosed, class III biological safety cabinets, and maintained under a negative pressure; extracted air from the cabinets and the laboratory is subjected to double HEPA filtration. The laboratory is dedicated to the reception, screening and analysis of such samples; that is, it is not used for other purposes such as research, because of the dangers of contamination of forensic evidence. In the UK, BSL-4 laboratories, at least at the present time, utilise cabinet lines contiguous with a terminal autoclave, rather than breathing air suits.

5. Samples received at the laboratory, after being collected, recorded and transported there, are opened within the class III glove box. The material within the sample to be analysed for biological agents, must be recovered as far as possible without disturbing other items in the sample, so as to maintain any other forensic evidence. For large volume samples, one aim of sample recovery is to reduce the volume such that it is suitable for further analysis. Once the material has been taken for analysis, the remaining items in the sample are safely stored to await the results.

6. Various initial screening techniques are available. Geiger counters and alpha particle counters are used to screen for radiological agents. As already mentioned, rapid radiological screening using Geiger counters is undertaken before the sample is opened. Further radiological screening is done at each stage during opening of the sample and recovery of material for analysis, to ensure the safety of the analysts.

7. Chemical detector paper and chemical agent monitors (CAMs) can be used to screen for chemical agents. For biological agents, there are limited screening options, although immunoassay test tickets or hand-held assays are available for the most likely agents to be used for BW and can be employed where there is a large enough amount of material in the sample. Whereas a negative result with CAM would be taken as indicative of the absence of chemical agent in the sample, a negative result with an immunoassay test ticket would not necessarily be taken as positive proof of the absence of biological agent, because of its lower sensitivity compared with, say, DNA-based analytical techniques.

Analysis of biological agents

8. In the UK, a range of techniques has been developed for the rapid and accurate analysis of high threat BW agents. In general, for viruses and bacteria these are DNA-based methods utilising rapid polymerase chain reaction (PCR). Antibody-based ELISA methods are normally used in the analysis of toxins, although rapid PCR may also have an application, where residual nucleic acid remains in the sample.

9. Confirmation of rapid analysis can be achieved in slower time using DNA sequencing (which is especially applicable to viruses) and conventional microbiological techniques such as culture, biochemical profiling, light and electron microscopy and, increasingly, mass spectrometry. Any sample showing a positive result in one test would be subjected to as many further analytical methods as possible in order to provide firm confirmation of the result. The "gold standard" for confirmation is culture of the BW agent, and the other microbiological confirmatory techniques are dependent on this initial step. The time taken for culture is specific to the organism involved: e.g., for *Bacillus anthracis* this may take between 24 to 36 hours; other agents may take significantly longer. Biochemical profiling using commercial tests (such as API) may require, for example, a further 2 days after a pure culture has been isolated.

10. The techniques used for DNA-based rapid and confirmatory analysis of biological agents require a number of performance characteristics, in particular high sensitivity and specificity, the ability to analyse complex samples, the ability to differentiate microbial strains or toxin variants, and the ability to analyse inactivated samples. Rapid analytical methods developed in the UK for BW agents are validated against the range of potential interferents that may be present in the variety of complex sample types (such as soil, water, vegetation, munitions, etc.) that may be taken during an investigation of the alleged use of BW.

11. For highly sensitive PCR assays in particular, which in the UK use fluorescence-based detection of amplified DNA for BW agent analysis, false positive results could arise through contamination of the test sample with BW agent DNA from spiked positive control samples. False negative results could arise from the presence of interferents of PCR in the complex sample (such as particles of clay, nucleic acid-destroying enzymes, etc.). Both these eventualities can be prevented by the incorporation of internal controls into the assay mixture. These internal positive controls utilise nucleic acid that is different to the target molecule and can be distinguished from it after amplification because the product uses a different detector dye, with a different fluorescence wavelength. If the test sample is negative, only the internal positive control DNA will be amplified, indicating that the assay is working correctly and no interferents are present. If the test sample is positive, both the BW agent DNA and the internal positive control DNA will be amplified. If interferents are present in the sample, the internal control DNA will not be amplified, indicating that alternative analytical methods must be employed.

12. For the future, biological mass spectrometry techniques have shown great potential for the confirmatory detection and analysis of toxins, viruses and bacteria. The extreme accuracy of techniques such as electrospray mass spectrometry in determining the molecular weight of biological molecules can allow proteins specific to particular BW agents to be identified in samples. Indeed, detection of minute differences between the molecular weights of a specific target protein in a BW agent can allow differentiation and identification down to the strain level. For example, the virulent Trinidad donkey strain of Venezuelan equine encephalomyelitis (VEE) virus and the attenuated VEE vaccine TC-83 strain can be identified and differentiated by this technique.

13. The ability to differentiate accurately and reliably between different strains of potential BW agents is an important consideration for analysis used in investigations of alleged use of BW. Strain differentiation can tell us something about the likely provenance of the agent, a key factor in any

investigation of alleged use. For example, some potential BW agents are known to occur naturally in the environment - though this may vary from region to region and there is generally little data available. This does mean, however, that an investigation team may have to consider the possibility that a positive result at the species level could be due to contamination from the environment rather than a positive indication of BW use. In this regard, quantitation of the amount of agent present in the environment, which can be done using quantitative DNA-based PCR techniques, would also be important – large amounts being more likely to be indicative of the use of BW than small amounts.

14. Furthermore, biological agents and toxins that are closely related to, or indeed the same as, those that may be potentially used for BW may be present in a facility or area for legitimate reasons, e.g., in the vicinity of a vaccine production plant. In order to resolve such ambiguities, and rule out the presence of a BW agent occurring naturally in the environment, analytical information beyond the species level may be required from some samples.

15. In the UK and elsewhere, DNA-based PCR analytical techniques have been developed to allow for strain differentiation of bacterial BW agents. These techniques have the added advantages that they are rapid, specific (to whatever level is required – genus, species or strain) and highly sensitive. They also do not require the agent to be viable in the sample, thus allowing analysis to be undertaken more safely, and with samples taken a significant time after the alleged event. Strain differentiation is achieved by exploiting the fact that different strains of particular bacteria often contain short sequences in their DNA that are repeated a variable number of times – the number of repeats being to some extent strain-specific. Targeting these variable number of tandem repeat (VNTR) regions in PCR analysis results in amplified products of different and specific molecular weights, and allows different strains to be distinguished and differentiated. It is possible to increase the resolution and accuracy of identification of particular strains and isolates of a potential BW agent by targeting more than one VNTR region in its genome. For example, work on this technique in the UK has allowed 19 very closely related strains and isolates of *Yersinia pestis* to be positively identified and discriminated. Such a technique also has an application in studying the epidemiology of naturally occurring disease.

Conclusions

16. Sampling and analysis have been identified as potentially important tools in investigations into the alleged use of BW, for example, under the auspices of the UNSG. Experience in the UK suggests that careful consideration needs to be given to the types of samples that may be taken, and the challenges they may pose to timely handling, screening and accurate and reliable analysis, and to the facilities and equipment in which such activities are undertaken.

17. Several useful techniques and methodologies are available, and under development, for analysis of BW agents in complex samples. Whilst these have great potential and utility, it is also important to recognise the potential limitations of sampling and analysis procedures, and to develop methodologies to minimise these. The development of fully validated analytical procedures, and the training and accreditation of analysts, are therefore important considerations in any efforts to strengthen the UNSG system for investigations into alleged use.

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