APPENDIX C

BIOLOGICAL AGENT DETECTION

Sampling and analysis for biological agents is a rapidly growing field. Many techniques and technologies are still under development. There are various factors to consider when sampling for biological agents, such as: method of dispersion for the agent, purpose of the sampling (e.g., to identify the agent, determine extent of contamination, confirm decontamination, etc.), environmental conditions, persistence of the agent, physical state of the agent, area/volume to be sampled, laboratory protocols, and others. It is important to note that biological agents (such as bacteria, viruses, and endotoxins) are particulate matter, and, therefore, detection methods are designed for particulate sampling. The following sections highlight some types of equipment that may be used for sampling and detection of biological agents.

A. SURFACE/BULK SAMPLING

1. Swabs

Swabs have been used frequently when sampling surface areas for the presence of biological agents. Swab tips come in a variety of materials, such as cotton, Dacron™, polyester, rayon, and foam. Shafts can be comprised of either wood or plastic. Generally, synthetic swab tips with plastic shafts are recommended because they are not of biological origin and will not interfere with DNA-based detection systems. Swabs may be used dry or wetted with a buffer solution. In general, studies have shown that wet swabs have higher collection efficiency than dry swabs.

2. Wipes and Sponges

Wipes and sponges are often used because they can sample larger surface areas and have a higher collection efficiency compared to swabs. They can also be used in a dry or wet fashion. Various styles and materials for wipes and sponges are available. As with swabs, synthetic materials are recommended to eliminate potential interference problems with detection systems.

3. Vacuum Methods

Vacuum methods can be used when it is necessary to sample very large surface areas or surfaces which are porous or irregular such as carpeting, where it is impractical to use swabs or wipes. These methods are also useful to gather bulk dust samples for analysis. One method utilizes a HEPA-filtered vacuum equipped with a dust collection filter sock which is used to capture the sample. Large surface areas can be vacuumed, and the dust gathered in the sock is then analyzed for the presence of biological agents. A similar method uses a portable sampling pump equipped with a filter cassette to "vacuum" particulate matter from smaller areas, and at lower flow rates. The filter can then be analyzed for biological agents.

4. Agar Plates

Agar plates, also known as "sticky plates," can be used to sample a surface by contacting the plate directly to the surface. The particles from the surface will adhere to the plate, which can then be analyzed by culture to identify any biological agents. This method has been used by various agencies during investigations of incidents involving biological agents.
B. AIR SAMPLING

Air sampling can be performed to determine the presence of airborne biological particulates. Essentially, a volume of air is drawn through a filter or deposited in another medium, and the captured particulates are then analyzed to identify biological agents. High flow rates are generally desirable because this allows higher sample volumes and increases the likelihood of detecting the suspect agents. However, it should be noted that some organisms are fragile, and the high velocities and impact mechanisms may kill the organism during the sampling process. Consult with SLTC to determine the appropriate flow rate and procedures for your situation.

Low flow air sampling methods consist of traditional personal sampling pumps equipped with capture devices such as filter media or liquid impingers. These low flow methods have the advantage of being small and portable; however, due to their low sample volume they will have a relatively high limit of detection.

Impactors, such as the Six Stage Viable Andersen Cascade Impactor, utilize higher flow rates (around 30 L/min), sample a greater air volume, and, therefore, increase the likelihood of detecting the agent. This and similar types of impactors capture the biological particulate directly on an agar plate which can then be analyzed in a laboratory by culture method.

High volume area samplers are also available for biological agents. These samplers possess flow rates ranging from 200 to 600 L/min, so they are able to sample very large volumes of air. Some instruments deposit the particulate matter on a filter, while others capture it in a liquid solution.

C. GENERIC DETECTION

There are several techniques and instruments available that will allow responders to perform a generic detection for biological agents. These methods will not identify a specific agent, but can be used to determine if a suspect material is of biological origin, and to rule out hoax materials. The following are some examples of equipment types:

1. **Particle Analyzers:** The particle size of a sample can be analyzed and compared to known size ranges for biological materials. If the particle size is too large or too small, biological materials can be ruled out.

2. **Fluorometer:** These instruments will detect the presence of DNA, which is a component of most biological materials. A positive response by the meter for a given sample indicates a biological material, but again, does not identify the material or agent.

3. **Luminometer:** A luminometer operates similarly to a fluorometer, except that it will detect the presence of adenosine triphosphate (ATP) in a sample. ATP is another component of a cellular organism, thereby indicating a biological material.

4. **Colorimeter:** Colorimeters can be used to detect protein from a sample. Again, protein is present in biological organisms, so these instruments can indicate if the material is biological in origin.

5. **Protein Paper:** Similar to a colorimeter, these paper strips can indicate if a given sample contains protein, and is, therefore, biological.
6. **pH Paper**: The pH of a sample is tested with pH paper strips; if the pH range is between 5 and 9, the material may be biological. If the pH is outside this range (below 5 or above 9), then biological materials can be ruled out.

D. **IDENTIFICATION**

1. **Immunoassay/Handheld Assay**

   An immunoassay test, also known as a handheld assay (HHA), can be performed on a sample to identify a specific agent. These HHA tests rely on an antigen/antibody reaction to identify the suspect agent. The test is presumptive, meaning that a given agent must be suspected and then tested with its specific HHA for confirmation. For example, if *Bacillus anthracis* (anthrax) is suspected, the sample is tested using an HHA designed for *Bacillus anthracis*; a positive result confirms the presence of the organism while a negative result indicates that the sample does not contain that specific organism. The HHA units are small, the test can be performed in the field, and they rely on a visual colorimetric change for sample results. Some HHA systems come with an electronic reader to aid in detecting the colorimetric change.

   HHAs are under scrutiny due to limitations on sensitivity and specificity; i.e., high rates of false-negative and false-positive results. The results from an HHA test should not be relied upon alone and further confirmatory analysis should always be performed. However, these tests are used widely by first responders as a rapid field test. Although they are presumptive, their results can assist decision makers in taking protective actions, treating potential infections, and involving other authorities as necessary.

2. **Polymerase Chain Reaction**

   Polymerase chain reaction (PCR) is a system that allows identification of an agent based on its DNA. The DNA from the sample is obtained and reproduced rapidly to produce a quantity that is detectable by the instrumentation. For example, after 30 cycles with the PCR system, one copy of DNA from an agent sample can be reproduced until there are one billion copies, which can then be analyzed and identified.

   PCR is performed real-time through detection by fluorescence. During the PCR cycle, DNA-specific "probes" with fluorescent dyes are attached to the DNA sample which allows detection. PCR can be performed in a laboratory, or in the field with semi-portable instrumentation. Specific reagents and supplies are necessary to perform the analysis.

   PCR has been useful for biological agent detection because it has excellent sensitivity, good specificity, and provides real-time results. Some weaknesses of PCR to consider are the following: potential interferences from other substances in the sample, reagent stability, and sample viability, because PCR will detect the presence of both live and dead organisms, but will not distinguish between the two.

3. **Culture**

   Analysis by culture is considered by many to be the "gold standard" for the identification of biological agents. Samples are sent to a laboratory where they are prepared and applied to an agar plate on which the suspect biological organisms are allowed to grow. After a sufficient period of time (usually 24 hours or more), visible growth can be examined to detect the presence of the biological agent(s). Often, culture is used for the confirmatory analysis of previous detection methods for a given sample (HHAs, PCR). Some disadvantages of culture include delayed results and the procedure will only detect living organisms. Any biological agent that has died before the analysis has begun will not be detected. Note that biological toxins or allergens associated with nonviable/nonculturable agents may still cause health effects.
**HRT Availability**

The following equipment is maintained by the [HRT](#) for use in biological agent sampling and analysis:

- Handheld Assays for the following agents: anthrax, plague, brucellosis, tularemia, Venezuelan equine encephalitis, staphylococcal enterotoxin B, botulinum toxin, ricin, smallpox, and Q fever.

- HEPA Vacuums: Two units with filter socks and other supplies specifically for use with biological agent sampling.

- Andersen Cascade Impactors: Two units.

- Dry Filter Units (DFU): Two units. The DFU is a high-volume air sampler designed for biological agent sampling. It operates at flow rates up to 600 L/min and utilizes a filter pad for capturing the agent.

- Surface/Bulk Sampling: Various wipe, sponge, and swab sampling kits.

NOTE: The HRT also serves as the coordinator for OSHA's biological SRT and can provide additional assistance and technical information regarding biological agent detection. Special precautions such as PPE and/or other work practices are also necessary to prevent exposure when working with biological agents. This information is provided as a reference for specially trained personnel and is not generally intended for CSHO use. Contact the [HRT](#) for more details.