

Appendix E: Medical Sample Collection for Biological Threat Agents

This guide helps determine which clinical samples to collect from individuals exposed to aerosolized biological threat agents or environmental samples from suspect sites. Proper collection of specimens from patients is dependent on the time-frame following exposure. Sample collection is described for “Early post-exposure”, “Clinical”, and “Convalescent/ Terminal/ Postmortem” time-frames. These time-frames are not rigid and will vary according to the concentration of the agent used, the agent strain, and predisposing health factors of the patient.

- Early post-exposure: when it is known that an individual has been exposed to a bioagent aerosol; aggressively attempt to obtain samples as indicated
- Clinical: samples from those individuals presenting with clinical symptoms
- Convalescent/Terminal/Postmortem: samples taken during convalescence, the terminal stages of infection or toxicosis or postmortem during autopsy

Shipping Samples: Most specimens sent rapidly (less than 24 h) to analytical labs require only blue or wet ice or refrigeration at 2 to 8°C. However, if the time span increases beyond 24 h, contact the USAMRIID “Hot-Line” (1-888-USA-RIID) for other shipping requirements such as shipment on dry-ice or in liquid nitrogen.

Blood samples: Several choices are offered based on availability of the blood collection tubes. Do not send blood in all the tubes listed, but merely choose one. Tiger-top tubes that have been centrifuged are preferred over red-top clot tubes with serum removed from the clot, but the latter will suffice. Blood culture bottles are also preferred over citrated blood for bacterial cultures.

Pathology samples: routinely include liver, lung, spleen, and regional or mesenteric lymph nodes. Additional samples requested are as follows: brain tissue for encephalomyelitis cases (mortality is rare) and the adrenal gland for Ebola (nice to have but not absolutely required).

Bacteria and Rickettsia

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
<p>Anthrax <i>Bacillus anthracis</i> <u>0 – 24 h</u> Nasal and throat swabs, induced respiratory secretions for culture, FA, and PCR</p>	<p><u>24 to 72 h</u> Serum (TT, RT) for toxin assays Blood (E, C, H) for PCR. Blood (BC, C) for culture</p>	<p><u>3 to 10 days</u> Serum (TT, RT) for toxin assays Blood (BC, C) for culture. Pathology samples</p>
<p>Plague <i>Yersinia pestis</i> <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture, FA, and PCR</p>	<p><u>24 – 72 h</u> Blood (BC, C) and bloody sputum for culture and FA (C), F-1 Antigen assays (TT, RT), PCR (E, C, H)</p>	<p><u>>6 days</u> Serum (TT, RT) for IgM later for IgG. Pathology samples</p>
<p>Tularemia <i>Francisella tularensis</i> <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture, FA and PCR</p>	<p><u>24 – 72 h</u> Blood (BC, C) for culture Blood (E, C, H) for PCR Sputum for FA & PCR</p>	<p><u>>6 days</u> Serum (TT, RT) for IgM and later IgG, agglutination titers. Pathology Samples</p>
<hr/> <p>BC: Blood culture bottle C: Citrated blood (3-ml)</p>	<hr/> <p>E: EDTA (3-ml) H: Heparin (3-ml)</p>	<hr/> <p>TT: Tiger-top (5 – 10 ml) RT: Red top if no TT</p>

Bacteria and Rickettsia

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
<p>Glanders <i>Burkholderia mallei</i> <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture and PCR.</p>	<p><u>24 – 72 h</u> Blood (BC, C) for culture Blood (E, C, H) for PCR Sputum & drainage from skin lesions for PCR & culture.</p>	<p><u>>6 days</u> Blood (BC, C) and tissues for culture. Serum (TT, RT) for immunoassays. Pathology samples.</p>
<p>Brucellosis <i>Brucella abortus, suis, & melitensis</i> <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture and PCR.</p>	<p><u>24 – 72 h</u> Blood (BC, C) for culture. Blood (E, C, H) for PCR.</p>	<p><u>>6 days</u> Blood (BC, C) and tissues for culture. Serum (TT, RT) for immunoassays. Pathology samples</p>
<p>Q-Fever <i>Coxiella burnetii</i> <u>0 – 24 h</u> Nasal swabs, sputum, induced respiratory secretions for culture and PCR.</p>	<p><u>2 to 5 days</u> Blood (BC, C) for culture in eggs or mouse inoculation Blood (E, C, H) for PCR.</p>	<p><u>>6 days</u> Blood (BC, C) for culture in eggs or mouse inoculation Pathology samples.</p>
<p>BC: Blood culture bottle C: Citrated blood (3-ml)</p>	<p>E: EDTA (3-ml) H: Heparin (3-ml)</p>	<p>TT: Tiger-top (5 - 10 ml) RT: Red top if no TT</p>

Toxins

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
<p>Botulism Botulinum toxin from <i>Clostridium botulinum</i> <u>0 – 24 h</u> Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays</p>	<p><u>24 to 72 h</u> Nasal swabs, respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays.</p>	<p><u>>6 days</u> Usually no IgM or IgG Pathology samples (liver and spleen for toxin detection)</p>
<p>Ricin Intoxication Ricin toxin from Castor beans <u>0 – 24 h</u> Nasal swabs, induced respiratory secretions for PCR (contaminating castor bean DNA) and toxin assays. Serum (TT) for toxin assays</p>	<p><u>36 to 48 h</u> Serum (TT, RT) for toxin assay Tissues for immunohistological stain in pathology samples.</p>	<p><u>>6 days</u> Serum (TT, RT) for IgM and IgG in survivors</p>
<p>Staph enterotoxigenesis <i>Staphylococcus</i> Enterotoxin B <u>0 – 3 h</u> Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays</p>	<p><u>2 - 6 h</u> Urine for immunoassays Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays</p>	<p><u>>6 days</u> Serum for IgM and IgG Note: Only paired antibody samples will be of value for IgG assays...most adults have antibodies to staph enterotoxins.</p>
<p>T-2 toxicosis <u>0 – 24 h postexposure</u> Nasal & throat swabs, induced respiratory secretions for immunoassays, HPLC/ mass spectrometry (HPLC/MS).</p>	<p><u>1 to 5 days</u> Serum (TT, RT), tissue for toxin detection</p>	<p><u>>6 days postexposure</u> Urine for detection of toxin metabolites</p>
<p>BC: Blood culture bottle C: Citrated blood (3-ml)</p>	<p>E: EDTA (3-ml) H: Heparin (3-ml)</p>	<p>TT: Tiger-top (5 - 10 ml) RT: Red top if no TT</p>

Viruses

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
<p>Equine Encephalomyelitis VEE, EEE and WEE viruses <u>0 – 24 h</u> Nasal swabs & induced respiratory secretions for RT-PCR and viral culture</p>	<p><u>24 to 72 h</u> Serum & Throat swabs for culture (TT, RT), RT-PCR (E, C, H, TT, RT) and Antigen ELISA (TT, RT), CSF, Throat swabs up to 5 days</p>	<p><u>>6 days</u> Serum (TT, RT) for IgM Pathology samples plus brain</p>
<p>Ebola <u>0 – 24 h</u> Nasal swabs & induced respiratory secretions for RT-PCR and viral culture</p>	<p><u>2 to 5 days</u> Serum (TT, RT) for viral culture</p>	<p><u>>6 days</u> Serum (TT, RT) for viral culture. Pathology samples plus adrenal gland.</p>
<p>Pox (Smallpox, monkeypox) <i>Orthopoxvirus</i> <u>0 – 24 h</u> Nasal swabs & induced respiratory secretions for PCR and viral culture</p>	<p><u>2 to 5 days</u> Serum (TT, RT) for viral culture</p>	<p><u>>6 days</u> Serum (TT, RT) for viral culture. Drainage from skin lesions/ scrapings for microscopy, EM, viral culture, PCR. Pathology samples</p>
<hr/> <p>BC: Blood culture bottle C: Citrated blood (3-ml)</p>	<hr/> <p>E: EDTA (3-ml)H: Heparin (3-ml)</p>	<hr/> <p>TT: Tiger-top (5 - 10 ml) RT: Red top if no TT</p>

Environmental samples can be collected to determine the nature of a bioaerosol either during, shortly after, or well after an attack. The first two along with early post exposure clinical samples can help identify the agent in time to initiate prophylactic treatment. Samples taken well after an attack may allow identification of the agent used. While the information will most likely be too late for useful prophylactic treatment, this information along with other information may be used in the prosecution of war crimes or other criminal proceedings. This is not strictly a medical responsibility. However, the sample collection concerns are the same as for during or shortly after a bioaerosol attack and medical personnel may be the only personnel with the requisite training. If time and conditions permit planning and risk assessments should be performed. Like in any hazmat situation a clean line and exit and entry strategy should be designed. Obviously, if one is under attack and in the middle of the bioaerosol, there can be no clean line. Depending on the situation personnel protective equipment should be donned. The standard Gas Mask is effective against bioaerosols. If it is possible to have a clean line then a three person team is recommended, with one clean and two dirty. The former would help decontaminate the latter. Because the samples may be used in a criminal prosecution, what, where, when, how, etc. of the sample collection should be documented both in writing and with pictures. Consider using waterproof disposable cameras, and waterproof notepads. Since, these items may need to be decontaminate. The type of sample taken can be extremely variable. Some of the possible samples are:

- Aerosol Collections in Buffer Solutions
- Soil
- Swabs
- Dry Powders
- Container of Unknown Substance
- Vegetation
- Food / Water
- Body Fluids or Tissues

What is collected will depend on the situation. Aerosol collection during an attack would be ideal, assuming you have an aerosol collector. Otherwise anything that appears to be contaminated can be either sampled by swabbing the item with swabs if available, or absorbent paper or cloth. The item itself could be collected if not too large. In the case of well after the attack collection samples of dead animals or people can be taken. In a manner similar to samples that would be taken during an autopsy. All samples should ideally be double bagged in Ziploc bags (the inner bag decontaminated with dilute bleach before placing in the second bag) labeled with time and place of collection along with any other pertinent data. If not use whatever expedient packaging is available that appears to reduce the chance of contamination of the sample and infection of personnel handling the sample.

These points are for consideration, since there may be little of no preparation time. If the agent can be identified from samples taken during, shortly after, or early post exposure either from exposed personnel or the environment; then measures to blunt the effects of the attack may be possible.