★ APPENDIX G

BIOLOGICAL SAMPLE/SPECIMEN COLLECTION AND MANAGEMENT

G-1. General

a. Critical elements for accuracy in analysis of biological samples and physiological specimens are correct collecting, packaging, handling, and transporting techniques. The quality of any analytical evaluation is directly related to the quality of the sample/specimen and the degree of postcollection degradation that occurs prior to testing. Combat health support personnel collect and submit specimens for suspect NBC hazards/agents involving humans and animals. Chemical corps and other nonmedical units collect and submit environmental (air, plant, and soil) samples for suspect NBC hazards/agents. Preventive medicine personnel collect and submit water and ice samples for suspect NBC hazards/agents. Veterinary personnel collect and submit food samples, such as fruits and vegetables; and specimens from animals for suspect NBC hazards/agents. Specimens collected by clinical laboratory personnel from inpatients at an MTF that are suspect of being exposed to a biological agent are forwarded to the supporting laboratory (such as the AML, theater Army medical laboratory, Navy land-based laboratory) personnel.

b. Essentially all military operations, (war, multinational deployments, military contingency operations, peacekeeping, peace enforcement, humanitarian support missions, and civic action programs) may generate some laboratory testing requirements. Each scenario, geographical region, population base, and suspect agent will impact on the type and amount of samples/specimens required and the collection process. During all operations, express permission is required before collecting specimens from civilians because of religious or sociological beliefs in many cultures. To obtain such specimens without permission could result in unnecessary mission complications.

NOTES

1. The term “sample” refers to nonhuman and nonanimal origin. The term “specimen” refers to human and animal origin.

2. Always consider that chemical agents may have been employed. You must check for chemical agents before collecting the sample/specimen. Chemical agents can damage or destroy biological agents. Also, chemical agents not identified in the sample/specimen can pose a hazard to receiving laboratory personnel. Mark all samples that are potentially contaminated with chemical agents as such.

3. Precautions should be taken to protect the sample/specimen collector from potential BW agents; at a minimum, respiratory protection and rubber gloves must be worn. Additional care must be taken when collecting samples/specimens to prevent cross-contamination. Gloves must be changed or decontaminated between sample collections.

4. Samples will not be delivered to the clinical laboratory of an MTF for analysis. They must be delivered to the designated supporting laboratory for processing. This will prevent the potential of accidentally spreading a biological agent in the MTF.

NOTES *(Continued)*

5. Prepare DA Form 4137 (Evidence/Property Custody Document). This form must accompany the samples/specimens from the point of collection to the final receiving laboratory. Each person receiving the sample/specimen must sign the document to provide evidence of chain of custody at all times.

c. Coordination for follow-on testing is absolutely critical to the sample/specimen collection process. The quantity, type, preservation, and acceptable delay in the time from collection to testing should be established before any sample/specimen is collected; otherwise, the effort may be compromised (to the detriment of both the individuals concerned and the authority awaiting the results).

d. Coordination with the receiving laboratory should be made to establish sample requirements, preferred collection techniques, methods of preservation, and transportation conditions, when the tactical situation and/or mission permits.

e. The number of individuals that need to be sampled varies with the type of analysis performed and the impact of the values determined.

- The number and types of individuals that need to be sampled, as well as the types of samples/specimens required, is determined by the analytical needs of the testing facility and the nature of the information required.

- Analysis to predict the prevalence, incidence, and impact of health hazards will be coordinated by the Epidemiology Section of the supporting laboratory. Their expertise and data analysis capability will guide the sample/specimen procurement process to best meet the requirements of each particular scenario.

- The number and types of "control" samples/specimens required to validate test information is determined by the supporting laboratory personnel. Random sampling, matched control populations, or other techniques will be employed as the requirements are identified.

- Confounding variables, interindividual variance, time-sequence significance, and other factors affecting analytical results are evaluated by the testing facility to predict their impact on results. The need to compensate for such variables is determined by supporting laboratory personnel; sampling methods are adjusted accordingly to mediate their impact as much as possible.

- Before submitting physiological samples/specimens to a laboratory for analysis, coordination with the receiving laboratory is essential. Most routine sample/specimen requirements can be verified through SOPs and submission manuals, but unusual or unique situations normally require direct contact with the testing laboratory to confirm the number and types of samples/specimens required to effect an accurate analytical process.

G-2. Sample/Specimen Collection and Preservation

a. Antemortem Specimens. Physiological specimens from living human or animal patients can include just about any conceivable body source or excreted by-product. It must be noted that

specimen types are seldom interchangeable; the exact type and amount of specimen required for a specific assay must be known before a collection procedure is initiated (see FM 3-19).

- Patients seen in an MTF may be a source for specimens suspected of containing biological agents. The primary medical care provider will determine the level of treatment for these patients and the specimens required for laboratory diagnosis. The MTF laboratory is not equipped to handle biological agents and therefore specimens generated will be forwarded to the supporting laboratory for analysis. Patient disposition will be based on evacuation policies, exposure, suspect agent, clinical symptoms, and required treatment/isolation.

- Blood specimens represent the most common analytical sample. Certain techniques and special care must be exercised to ensure an acceptable specimen is collected and to minimize an adverse affect to the patient or specimen collector. In general, phlebotomy requires the use of a 20 to 22 gauge needle to minimize mechanical hemolysis during aspiration using a syringe or Vacutainer™ tube collection system. Blood collected with a syringe and needle should be transferred to an appropriate Vacutainer™ tube immediately after collection. The type of tube, type of anticoagulant or preservative, and amount of blood collected will vary with the specific assay requested. Unless some special sample preparation step is required, the blood is best left in the original rubber-stoppered tube for transport.

- Urine specimens are best collected using a clean-catch (midstream, if possible) technique in a sterile urine cup. The volume of sample required will vary depending on the specific assay requested; however, 25 to 50 ml is sufficient for most tests.

- Tissue specimens can originate from any body source accessible by scrapping, swabbing, or minor excision. Tissue specimens are collected by medical personnel trained for this task. Specific techniques for collecting these specimens are not provided in this appendix.

NOTE

In cases where the supporting laboratory cannot be contacted, as a minimum the following specimens should be collected: Urine—25 to 50 ml in a sterile container. Blood—two 7 to 10 ml tubes without anticoagulant (red-stoppered Vacutainer™); two 7 to 10 ml tubes with potassium or sodium EDTA (lavender-stoppered Vacutainer™).

- All specimens (regardless of physiological source) must be labeled to positively identify the individual or animal from whom it was collected; at a minimum, the individual's full name, unique personal identification number (SSN when possible), military unit and location, and date and time of collection should be written on the label of the specimen container.

- All specimens are collected using aseptic techniques. All specimens are packaged, handled, and transported in a manner that ensures they arrive at the final destination laboratory in a testable condition. Personal protection guidelines must be adhered to when collecting or processing specimens; at a minimum, this includes gloves and a mask. In the laboratory, a gown or other protective items may also need to be used. In the field, under suspect NBC conditions, collectors

should be in MOPP4 or inside NBC-protected vehicles. Common sense and the clinical and/or tactical situation will determine the extent of personal protection necessary.

- Preservation of specimens, either chemically or mechanically (cooling), will be necessary to minimize the amount of analyte degradation that occurs after removing the specimen from its physiological microenvironment. The optimal preservation technique will vary with different laboratory tests and must be confirmed for each requested assay. While freezing may preserve some serum constituents, freeze-thawing cycles may denature others. Freezing may also completely destroy certain microorganisms. This caution also applies to tissue specimens since "fixing" tissue with a standard 10 percent formalin solution will preserve tissue for special staining techniques; however, it renders the specimens completely useless for microbiological culture. Always verify specimen preservation requirements for storage and transport with the supporting laboratory before processing the specimen. Ideally, confirmation of the correct handling conditions should be coordinated before collection.

- The importance of coordinating sample/specimen collection with the supporting laboratory facility cannot be overstated. Contact the receiving laboratory for instructions when doubt exists about the appropriate source, collection technique, storage and preservation conditions (such as, aerobic or anaerobic environment), and transportation requirements for samples/specimens. Extremely small volumes of samples/specimens, properly collected and handled, can yield a tremendous amount of information to assist in making medical, tactical, and strategic decisions. Conversely, very large quantities of poorly collected and insufficiently preserved samples/specimens are essentially worthless for most analytical techniques.

- Analysis beyond intratheater capabilities will be coordinated by the supporting laboratory, when deployed, or through medical channels in the absence of an in-theater supporting laboratory.

b. Postmortem and Forensic Specimens. The analysis of specimens from deceased humans and animals can provide valuable information about the disease, organisms, injuries, or environmental conditions at the time of death. This information can greatly enhance the treatment of others affected by the same, or physiologically similar, process. Specimen collection for postmortem or forensic examination is very important; the techniques involved reflect a significant degree of training, experience, and skill.

(1) The collection of specimens from remains should be conducted exclusively by a pathologist, or other personnel specifically trained in forensic collection techniques. An exception is when SOF personnel are operating under radio silence conditions; the most qualified medical person with the operation collects, preserves, and transports or coordinates transport of specimens for evaluation. The same chain of custody requirements apply to specimens collected by SOF personnel, as with all other specimens.

(2) A large amount of support information can be gained by analyzing the site of injury and subsequent death. This "site scene" investigation requires a tremendous attention to detail and a trained observer. If forensic personnel cannot be contacted, or will be unduly delayed in arriving at the scene, then photographs of the victim and the immediate surroundings should be made. The scope and extent of the photographs should be composed to reflect as much detail as possible to assist forensic personnel in reviewing the scene retrospectively. In the event that photography is not feasible, detailed sketches of the scene should be made to assist the forensic investigation.

(3) Techniques such as cardiac or bladder puncture, needle biopsy of organs, spinal tap, or exploratory laparotomy should not be performed by untrained personnel unless specifically requested and directed by forensic investigators.

c. Water Sample Collection.

(1) Water samples for identification or verification of biological agent contamination are collected by PVNTMED personnel. The supporting laboratory should provide guidance on sampling procedures and collecting kits for use in collecting the samples. In the absence of guidance, a technique for use of the Sep-Pak is described in FM 3-19.

(2) When sampling kits are not available, samples may be collected in other available containers. The containers must be sterile. The best containers for use are the 100 ml glass bottles used for collecting routine water samples. All water samples must be collected and placed in a cooler or refrigerator until the sample is transported to its destination. During transportation the samples must be maintained at a temperature between 1^0C and 4^0C.

d. Food Samples. Veterinary personnel must collect suspect biologically contaminated food samples for submission to the supporting laboratory for in-theater verification of contamination. All food samples must be collected and placed in sterile containers. Place the samples in a cooler or refrigerator until the sample is transported to its destination. During transportation the samples must be maintained at a temperature between l^0C and 4^0C.

e. Environmental Samples. Environmental samples are collected as directed in the operators' manual or other publications for operating collection systems. Example: The Biological Integrated Detection System (BIDS) collects an environmental sample using a single liquid sample collector. The collector is a high-volume aerosol sampling and collection device. On demand it samples ambient air through a two-stage virtual impactor which concentrates aerosol particles in the 2 to 10 micrometer diameter size range. The concentrate particle stream is directed through a wet collector containing a buffer solution and over a 45 minute period a 40 to 50 ml sample is collected. On order or when test results indicate a suspected agent, the sample and associated documentation are packaged and transported IAW FM 3-101-4, Appendix H (Biological Detection Platoon Operations; Sampling, Handling, and Chain of Custody).

f. Chain of Custody.

(1) A strict chain of custody must be maintained for every sample/specimen collected. Use DA Form 4137 (Evidence/Property Custody Document) for each sample/specimen collected. The DA Form 4137 must accompany the sample/specimen during transport from the point of collection to the final receiving laboratory. Each time the sample/specimen is transferred to another individual, the receiving person must sign the document to show that they received the sample/specimen. The document will provide the answer to the following questions:

- When was the sample/specimen collected?
- Who has maintained custody of the sample/specimen?
- What has been done with the sample/specimen at each change of custody?

(2) The samples/specimens must be appropriately packaged, labeled, and evacuated to the designated laboratory for confirmation of a biological attack. The standard chain of custody for the evacuation would be as follows:

- Sampling unit.
- Unit S2 or medical operations officer.
- Technical Escort Unit or other command-designated escort personnel.
- In-theater laboratory, if in operation.
- Technical Escort Unit or other command-designated escort personnel.
- CONUS laboratory.

G-3. Sample/Specimen Transport

Samples/specimens submitted for laboratory analysis must be properly packaged, labeled, and shipped to ensure they arrive in an analytically acceptable condition. All samples should be maintained at a temperature of 1°to 4°C during transport. Ideally, samples/specimens should arrive at the in-theater laboratory within 6 hours of collection. The samples/specimens should be delivered to the CONUS laboratory within 24-48 hours. If the samples/specimens cannot be delivered to the CONUS laboratory within this time, then they should be flash frozen to -165°C, if capabilities are available. Dry ice should be used when flash freezing cannot be accomplished, if available. If the samples/specimens cannot be delivered to the CONUS laboratory within 24 hours, the supporting laboratory should subculture the samples/specimens. The supporting laboratory should send the subculture with the samples/specimens to the CONUS laboratory. The subculturing date should also be provided.

a. Coordination must be made with Technical Escort Unit personnel for transporting the samples/specimens to the laboratory. When these personnel are not available, a chain of custody must be established and personnel designated with the responsibility for escorting the samples/specimens to the supporting laboratory and on to the CONUS laboratory. Regardless of who escorts the samples/specimens, a documented chain of custody must be maintained.

b. All samples/specimens should be sealed in plastic bags, or other containers to prevent leakage during transport. The containers must contain sufficient absorbent material to absorb the entire contents in the event of a leak. This minimizes the risk of contamination to escort and laboratory personnel. The sample/specimens must be packaged in an International Air Transportation Association (IATA) -approved sample transport container for shipment/delivery to the CONUS laboratory. If an IATA sample transport container is not available, ice (wet or dry depending upon required temperature) may be used for initial packaging and transport in-theater. However, the ice must not be in direct contact with the samples/specimens; place the ice in plastic bags, or other such material, to cool the samples/specimens during transit. Conversely, the samples/specimens (or packing container) may need to be insulated to minimize temperature extremes during shipping. However, for-transport out of theater, the samples/specimens must be packaged in an IATA-approved container.

c. The chemical composition of samples/specimens can rapidly deteriorate when exposed to excess heat, solvents, sunlight, and even ambient air. Likewise, microorganisms may not survive even relatively short periods of time if not kept at the correct temperature and, optimally, contained in a supportive transport medium. Instructions for packaging and transport conditions should be

obtained from the supporting laboratory facility. Special transport medium, if necessary, is requested from the supporting laboratory when it is not readily available to the collecting unit.

d. As mentioned previously, accurate and complete sample/specimen identification is necessary. An adhesive label on the primary sample/specimen container is best, along with a list of all samples/specimens packaged in a secondary transport container, if more than one sample/specimen is being shipped.

e. A short written narrative of the facts, circumstances, and conditions generating the samples/specimens should be included with the shipment. Include the collector's name, unit, and telephone number (if available), and the grid coordinates of sample collection point. This provides useful information to the laboratory investigators; it can guide them in expanded or follow-on testing.

f. Samples/specimens will not be split prior to arrival at the first receiving laboratory. The initial receiving laboratory will extract the required amount (an aliquot) of sample/specimen for their use. The laboratory is equipped to extract portions of the sample/specimen while preventing cross-contamination (introduction of contamination at the time of splitting) of the sample/specimen. This will ensure that valid samples/specimens arrive at the CONUS laboratory for confirmation of use of biological warfare agents; thus providing the National Command Authority with required confirmation.

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