Ad hoc Group of Governmental Experts to Identify and Examine Potential Verification Measures from a Scientific and Technical Standpoint

Distr.
RESTRICTED

BWC/CONF.III/VEREX/WP.57
25 November 1992

ENGLISH only

Second Session
Geneva, 23 November - 4 December 1992

UNITED STATES OF AMERICA

Biological Sample Collection, Preservation and Transportation

Summary: This paper provides a description of methods for obtaining, preserving, securing, and transporting samples of potentially highly toxic and pathogenic biological materials regarded as analytically important. It is intended to serve as a guide for collectors since techniques must be adapted to each specific sample.

This discussion does not evaluate sampling as a verification measure. It is understood that the relative merits of sampling as part of the verification measure of on-site inspection will be subjected to examination by the ad hoc experts group in accordance with the six criteria of the group's mandate. Moreover, it is assumed that this examination will occur within the context of the three functional areas of (1) research/development, (2) production, and (3) weaponization/stockpiling. The descriptions provided in this paper are designed to help the group define what is meant by the term "sampling" so that the examination of sampling and its potential role in an on-site inspection may proceed from a common understanding.

Introduction: A critical assumption in technical discussions continues to be that analytical samples obtained under a wide variety of conditions will be subjected to a wide variety of analytical techniques. Hence, an analysis can be no better than the sample; the selection, sampling techniques, containment, and preservation of the sample during transport. A documented descriptor of the sampling operation, a documented chain of custody and audit trail, a safe and tamper-resistant transportation container are vital to the integrity of the sample and the subsequent laboratory analysis.

Before discussion of the details of sample collection, preservation and transportation, there are some related issues which should be considered by the experts. These generally involve the utility of sampling and subsequent analysis to determine compliance. For example, sample locations must be carefully chosen whether inside the facility or equipment which is suspected of non-compliance. The problem of sampling blood of personnel and laboratory animals requires legal, health and analytical consideration. Similarly, sampling of munitions can pose serious safety and environmental problems. Are there techniques available to safely sample these items? Are there non-destructive techniques available which would preclude physical sampling? Indeed the basic question must be answered before any sampling is done. Can we adequately analyze samples in the field to provide satisfactory evidence of

non-compliance? Are there on-site techniques available which are reliable enough to prevent false positives or must we rely on off-site laboratories and transport of samples to satisfy concerns? With these types of concerns in mind the following discussion provides information on specific sampling techniques which have been developed.

Audit Trail: A documented audit trail and chain of custody is required for any sample taken or identification technique used. Items which should be included are:

- Geographical location of sampling to include installation site, city, county, country, longitude and latitude, as appropriate.

- Specific location of sampling to include building or enclosure location. In a building or facility, identify exact location of tank or drain relevant to process operations. In an open area, the exact location of vapor, soil, water or artifact sample should be related to the proximity of a facility or other fixed installation. The signature or initials of the sampler are required.

- Identification of the samples taken is to include the number of duplicate samples taken, the aggregate amount of sample taken and the partitioning of any samples with the number of samples resulting from the partition of any sample. A specific code number should be affixed to each sample resulting from the operation to allow tracking the samples through the analysis process. The signature or initials of the sampler are required.

Samples from Facilities: All samples should be taken in accordance with an agreed-upon plan designed to protect confidential business information regardless of the location. Samples to monitor a declaration, support a material balance determination or resolve anomalies should be analyzed on-site if possible and if feasible. All samples should be taken by, or in the presence of the sampling team. Samples should be sealed and labeled by tamper-resistant methods that ensure sample identity and maintain a custody chain until analysis is completed. Sampling procedures should attempt not to interfere with routine facility operation nor affect the operating safety of the facility.

Bulk Product Samples: Within the site, samples should be taken from both temporary and permanent bulk product storage areas. At a minimum, samples should be taken from labeled unit containers. Product samples could be liquid or dry dependent on the processing stage.

Waste and byproducts: Samples should be taken from waste or byproduct streams, holding tanks or impoundments used in the waste system. Waste stored for off-site shipment should also be sampled. In addition, HEPA filters from cabinets or room areas should be sampled and wipe samples taken from ventilation and processing areas.

Raw Materials: Samples, liquid or dry, should be obtained from drums, tank cars or trucks, portable containers as well as fixed storage, holding tanks or fermentors to confirm the composition and intended use.

Products: Both in-process and final product samples should be taken from the production line before final packaging and storage.

Sampling techniques: The sample should be taken with a non-contaminated sampling device. If it is taken through a fixed, existing sample port or line, the sample path should be flushed with the material to be sampled to ensure that the sample is free of any prior contaminants. The flushed material should be saved, since if a violation occurred, the violator may have forgotten to flush the sample line before he changed the contents of the containment vessel.

The sample should be captured in a suitable clean, contamination free teflon or glass container that is numbered, tared, and can be sealed immediately.

The samples should be properly identified by sample site, the time of day, date, month and year, who took the sample, how it was taken, and a brief description of the material (color, physical form, etc.). The data on the sample should be entered on the numbered label and on an accompanying sample log along with the name and signature of both the sample taker and the inspector who witnessed the sample being taken.

The size of the sample and the number of samples should be determined by the need for analysis by the collection team, by the facility personnel, and retention sample for recheck if required. If simultaneous samples can be taken, then allocation of the samples can be made at the time of sampling. If only one sample can be taken, its size should be such that division of the sample will not be a cause for dispute. The division should take place at the collection site in the presence of the collector and the site representative.

The sample to be removed should be appropriately sealed and placed in a container using internationally recognized methods for packaging and transportation. Each sample should normally be placed in its own mylar bag, although similar samples may be grouped together in the same overall bag if cross-contamination can be avoided. The bags should then be placed in a portable cold storage chest which can also be sealed, tagged, labeled and secured for transport in a manner designed to preserve the chain of custody. A record of the samples and the cold box container numbering and coding system should also be kept in the inspector's notebook. It is important to be able to have a custody record from the time the sample is taken until it has been analyzed at the off-site analytical laboratory. The custody record should be continued in the laboratory to ensure accurate results. Unused samples should be returned to the host country and if possible observers should witness sample analyses.

Seals should be broken only in the presence of an authorized collection team representative. If safety or other considerations make it necessary, at any time, to break the seal on a sample-containing mylar bag, or cold box, a written record should be kept as to who broke the seal, why it was broken, and how long a period elapsed before resealing and who resealed the material. Resealing should occur as soon as possible and new seals or tag numbers entered into the record book. The old seals should be retained and accompany the material to the laboratory for analysis.

Sampling equipment: This should include, but is not limited to the following items:

Collectors: clean bottles, vials, gas tubes, tubes, vacuum bottles, etc. The size and number of each container should be determined by the nature of the sample. All sample containers should be numbered, clean, durable, sealable, and be tared where possible.

Sampling Aids: These should include syringes, liquid and solid waste samplers, core or thief samplers, etc. The collection team may also need to carry supplies of non-contaminated or sterile distilled water, reagent grade solvents and containers to clean the sampling equipment and decontaminating solutions. There should be a sufficient amount of labels, receipts, stickers, seals, recording notebooks and other record-keeping documentation as appropriate.

Sample storage/transport: should include numbered containers which can be secured for sending sets of samples to a controlled and secured on-site laboratory.

The samples to be transported for off-site analysis should be packaged as described above in safe/secure refrigerated containers according to safety product information (include in container) and shipped in accordance with pertinent environmental, public health and agricultural regulations to the off-site laboratory for analysis. At the laboratory the samples will be unpacked, identified and weighed in accordance with the procedure for chain of custody of samples and confirmed with the identification provided by the collector before storage to preserve sample integrity. The material courier receipts will be documented to assure sample control.

Tags and Seals: Sealing and identification devices must be simple, relatively inexpensive and unique on the basis of features easily and permanently fixed to the sample container. Confirmation of the authenticity and the integrity of the seal should also be relatively simple. In many existing and proposed systems, the seal is simple but the method for authentication is prohibitively complex. The following candidate methods preserve identity of the samples from collection to analysis and satisfy the problem of keeping track of samples in transit. Three-dimensional reflective systems, passive interrogation systems with unique identity, methods that exploit biological specificity, and methods for permanently affixing tags and seals are relatively mature technologies that can be incorporated into sampling and transport schemes to ensure integrity.

Sample Sources: Samples containing biological agents or other pathogens can be divided into environmental samples (vapors or aerosols, liquids, soil, vegetation, munitions or dissemination devices, used ordnance, etc.) and biomedical samples (urine, blood, sputum, organs, tissue biopsies, etc. from acutely ill or dead casualties). In suspected use situations, background samples from "clean" areas should be taken by identical methods to provide a baseline. Personnel collecting the samples should be wearing appropriate protective equipment and should be decontaminated following the sampling operation.

Environmental Samples: Methods for collecting environmental samples will depend on the sample type and the conditions affecting the collection, e.g., weather, age, soil type, etc. The samples must be documented, preserved and transported as described earlier to provide the same assurances of authenticity as any declared specimens. Sample collection methods are illustrated by the following:

Liquid aerosol/vapor samples: An electric or hand pump should be used with care to record the approximate volume of the sample and the type of collection tube or concentrator used should be recorded.

Vegetation samples: Collect material from several locations within the area taking care to preserve deposits of dust, etc. The sample size should be several leaves or handfuls of grass.

Soil samples: Collect samples from suspect areas with similar samples taken beyond the perimeter of the area. Sample volume should be approximately that of a cigarette pack lying on its side.

Liquid samples: Take samples from standing pools or along streams where contamination is suspected. Bulk water samples are collected by skimming surface water into a collection bottle or the use of resin collection filter columns.

Munition or dissemination devices: The suitability of non-destructive methods for biological munitions has not been evaluated nor have methods been developed to nonintrusively differentiate biological from chemical or conventional munitions. Opening a biological item may be extremely hazardous especially if the fill is unknown and will require special containment equipment. The questions surrounding sampling of these items need to be fully explored.

The samples should be thoroughly documented and refrigerated or chilled immediately. Do not freeze. Small animal samples are packaged in the same manner as other samples. Ordnance or remnants of munitions and protective clothing or equipment constitute important sources of biological agents for identification purposes. Only qualified ordnance experts should collect such items but the general procedures for collection and packaging are the same as for other materials.

Biomedical samples: The best biomedical sample in a use investigation is an acutely ill individual or a cadaver. Sample documentation forms and symptoms are to be completed on all biomedical samples. Once collected, the samples should be refrigerated or chilled immediately. Do not freeze. Medical personnel should perform biomedical sample collection to ensure that a valid sample is obtained and to ensure accurate description of symptoms. The following is guidance for sample collection which can be modified according to clinical findings and suspicions.

- Collect samples from patients during acute phase and at day 7.
- Collect urine samples (20-50 ml per sample x 3) in urine specimen cups, secure the top with wide parafilm tape and place in individual sealable mylar bags.
- Collect whole blood or serum samples (5 ml per sample x 3) in red-top blood tubes and place in individual mylar bags.
- Collect sputum only from acutely ill patients (x 3). They should be collected in urine cups and sealed as above.
- Collect cerebral spinal fluid (2 ml per sample x 3) in red-top blood tubes and secure as above.
- Take at least 30 grams of organs/tissues (human, post mortem x 3), place in a sterile container in individual, sealable mylar bags and refrigerate immediately; liver, spleen, lung, subcutaneous fat, kidney, heart and brain.
- Collect at least two mediastinal lymph nodes post mortem.
- Photographs and questionnaires should be employed where appropriate.

For confirmation of immunization records and occupational safety and health records it has been proposed by some to collect whole blood or plasma samples from operating personnel or laboratory animals. This represents a special case and if deemed necessary, will require extensive negotiations to reach agreement on their value and to resolve both the legal and the confidentiality issues as well as insure that public health and safety issues are fully considered.

Packaging and transport of biomedical samples to an off-site laboratory for analysis should follow the same rigorous procedures for documentation and ensuring the chain of custody as previously described. If analysis is normally performed off-site, authenticity of the samples is especially important.

General: Issues such as confidential business information must be addressed before sampling and analysis can be completed. Can proprietary information be adequately protected throughout the analytical process and the subsequent reporting process as well as during the sampling where, for example, special media for cost effective production may be disclosed?

Although not a part of sampling, the feasibility of on-site analysis may affect the sample processing and handling. Little study has been completed as to the availability of analytical technology which is portable, accurate and reproducible. Do such tests exist for all of the possible classes of bacteria viruses, rickettsia, toxins and genetically modified materials? Are reagents stable enough to reduce the logistical burden? What does PCR technology offer? Are the available tests insensitive or inappropriately specific? Should broader tests be developed for use on-site? Is there any way to detect genetically modified organisms on-site? Can analytical work be performed safely in on-site facilities or is special containment required for safety? These are but a few of the many questions which come to mind which need careful, complete analysis and resolution.

Conclusions: Representative procedures for sample management that could allow for credible analysis have been developed and described here. A set of procedures must be developed and standardized which will be usable for all potential circumstances. Such procedures are necessary to provide confidence in the overall system of collection, transportation and analysis. The foregoing discussion presents a number of factors that need to be considered in developing the procedures, and in some cases, suggests procedures that might be utilized. A number of questions remain to be resolved.

A forecast of changes due to technological developments over the next five years indicates that only instrument advances to allow comprehensive on-site analysis would make any difference in sample packaging and transportation procedures. Sample collection procedures would remain constant even if on-site analysis were possible. The use of sophisticated instruments on-site is not believed possible in the five to ten year timeframe, and development of simple "yes or no" tests for specific agents is progressing slowly. Overall, few significant changes are expected in this area in the five year and perhaps in the ten year timeframe which would make sampling simpler, cheaper, or more complete.