UCRL-AR-143245

Sampling Requirements for Chemical and Biological Agent Decontamination Efficacy Verification

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March 29, 2001

U.S. Department of Energy

Lawrence
Livermore
National
Laboratory

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This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

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1. Introduction

This document describes procedures and protocols that are currently available that can be used to verify the efficacy of decontamination procedures after a civilian terrorist event using chemical warfare (CW) or biological warfare (BW) agents. The document describes protocols for the collection of CW and BW samples, as well the creation of the sampling design.

1.1. Why sample?

A suspected or known release of a CW/BW agent by terrorists on a civilian target will trigger a chain of events in responding to the release. First, a release of an agent on a target is either suspected (through advertised threats or other intelligence) or known (through detection of the agent or observed casualties). Second, the affected area is secured by emergency response personnel (usually through evacuation and access control). Third, the area will be either decontaminated or in some way rendered safe for resumed human use. Fourth, the area will be released from access control. Obtaining samples to determine the presence of an agent may be necessary to:

- Verify an agent release has occurred
- Identify areas and surfaces requiring decontamination
- Determine handling and disposal procedures of expendable items
- Verify decontamination efficacy
- Monitor natural attenuation

In the event of a suspected release of an agent in the absence of obvious casualties, it may be desired to verify the agent's presence. This will most likely be conducted by broad-area air sampling. It may be necessary to more precisely define the areas and surfaces contaminated with agent to more efficiently plan the decontamination process. or This could involve the collection of surface swab samples, or, in the case of CW agents with a significant vapor pressure, the collection of point air samples using a hand-held air sampler such as the CAMs device used by the Army. It may be necessary to determine the presence of CW/BW agent on items deemed not worthy of extensive decontamination (such as draperies, carpets, etc) to allow for safe handling and proper disposal of the items. Once an area has undergone decontamination, sampling will be necessary to verify the decontamination procedures and that the area is safe for continued human use. Finally, under certain circumstances, it may not be necessary to undertake decontamination actions if environmental conditions suggest the agent will

degrade naturally into non-toxic substances. In this case, sampling may be warranted to monitor the natural attenuation process.

1.2. What should be sampled?

The Department of Energy (DOE) Chemical and Biological Nonproliferation Program (CBNP) has developed three civilian "scenarios" of incidents to guide the CBNP in developing decontamination technologies to respond to civilian terrorist activities. The scenarios are:

1. An outdoor location: includes sports stadiums, public park areas such as the Washington D.C. Mall, airport runways, and airplane exteriors.
2. A semi-enclosed location: includes facilities such as convention centers, subways and airport terminals.
3. An indoor location: includes airplane interiors, historic buildings such as the U. S. Capitol or the White House, important business locations such as the New York Stock Exchange, and buildings at Department of Energy and Department of Defense facilities housing strategic materials. Potential long-term residence in affected buildings is part of this scenario.

A common characteristic among these scenarios is that each is of sufficient importance, either economically or historically, that the public will desire reuse of the area and therefore the expense of decontamination is warranted. However, even within these scenarios, there will be items and materials for which it is more efficient to remove and dispose in lieu of decontaminating. Examples of items most likely to be removed and disposed of include carpeting, draperies, common office or building furniture, other removable floor coverings, replaceable documents, etc. However, it may be necessary to sample these items (or a composite of the items) to determine safe handling and disposal procedures.

Items most likely to be retained and thus decontaminated within each scenario include, but are not limited to such things as structural components of buildings, high-value equipment, culturally important landscaping, historical documents and valuable artwork. As a consequence, materials that may require decontamination and subsequent sampling may include soil, grass, landscaping, natural and semi-natural water ponds, concrete, asphalt, and structural building materials such as wood, glass, marble, brick, plaster tile, glass, painted and unpainted metals, cinderblock and rubber. Specialized equipment that may require decontamination includes ventilation systems, elevators, escalators, specialized electronics, and emergency life support equipment. The decontamination of historically significant artwork and important records would

involve materials such a painted canvas and paper. This diverse array of materials and surface geometry will likely require a suite of sampling techniques. This report is intended to provide some of the protocols and techniques that may be used in developing a decontamination verification sampling program.

2. Collection of Samples for Chemical Agent Analysis

2.1. Purpose

This chapter describes procedures for collecting samples of various matrices for the purpose of analyzing the sample for the presence of chemical warfare (CW) agents. Chemical warfare agents can be classified on the basis of a number of physical and chemical properties. Table 2.1. lists the major chemical agents. The procedures described in this chapter are intended for use during decontamination activities in a civilian scenario, that is, for decontaminating a civilian target after the release of CW agents at that target. Although intended to be used in a civilian context, the information presented in this chapter draws heavily upon procedures and protocols developed in support of the Chemical Weapons Convention (CWC) and by the U. S. Army for military decontamination activities. In addition, we utilized appropriate protocols and procedures developed by the U. S. Environmental Protection Agency (EPA), particularly with respect to chain-of-custody, sample handling, and quality control/quality assurance requirements.

2.2. Sampling Plan

A sampling event begins with the creation of a sampling plan. The sampling plan should contain the following: 1) project objectives, 2) data quality objectives, 3) sample collection requirements, 4) analysis and testing requirements, 5) quality control requirements, 6) required project documentation, and 7) identification of the organizations conducting laboratory and field operations (USEPA 1997). The creation of sampling plan should not be treated lightly, for the amount of logic and analysis used to create the sampling plan will largely determine the acceptability of the results by various stakeholders. While it is recognized that speed is an important consideration when conducting decontamination verification activities, it is important the above seven items be considered and documented.

The sampling plan should contain a clear statement of the objectives of the sampling event (the project objectives). This includes a statement of what problem is to be solved (such as certifying a building safe for re-occupancy) and the information required in the process. Data quality objectives (DQOs) describe the overall level of uncertainty that the decision-make is willing to accept in the results derived from the sampling event. DQOs should be set for both acceptance of the analytical results as valid and appropriate, as well as accepting that an area as been adequately decontaminated.

Table 2.1. Chemical Warfare Agents

Agent Class	Agent	Symbol	Persistency	Rate of Action
Nerve	Tabun	GA	Low	Very rapid
	Sarin	GB	Low	Very rapid
	Soman	GD	Moderate	Very rapid
	GF	GF	Moderate	Very rapid
	VX	VX	Very high	Rapid
Blister	Sulfur mustard	H, HD	Very high	Delayed
	Nitrogen mustard	HN-1	High	Delayed
		HN-2	Moderate	Delayed
		HN-3	Very high	Delayed
	Phosgene oxime	CX	Low	Immediate
	Lewisite	L	High	Rapid
	Phenyldichloroarsine	PD	Low-moderate	Rapid
	Ethyldichloroarsine	ED	Moderate	Delayed
	Methyldichloroarsine	MD	Low	Rapid
Choking	Phosgene	CG	Low	Delayed
	Diphosgene	DP	Low	Variable
Blood	Hydrogen cyanide	AC	Low	Rapid
	Cyanogen cyanide	CK	Low	Rapid
	Arsine	SA	Low	Delayed
Riot control (vomiting)	Diphenylchloroarsine	DA	Low	Rapid
	Diphenylcyanoarsine	DC	Low	Rapid
	Adamsite	DM	Low	Rapid
Riot control (tear gas)	Chloroacetophenone	CN	Low	Immediate
	Chloropicrin	PS	Low-high	Immediate
	Bromobenzylcyanide	CA	Moderate-very high	Immediate
	O-chlorobenzylidene malononitrile	CS	Low-high	Immediate
Psychochemicals	3-Quinuclidinyl benzilate	BZ	High	Delayed

From The Biological and Chemical Warfare Threat. Full citation unavailable.

Sample collection requirements include sampling procedures, locations, equipment, holding times, preservatives and field quality assurance requirements. Analysis and testing requirements include a list of analytes to be analyzed for, analytical and testing procedures to be employed, required detection limits, and requirements for precision and bias. Quality control procedures should be specified for estimating the precision and the bias of the data. Project documentation requirements include identifying the documents and data that must be maintained. Project documentation should be sufficient to allow review of all aspects of the work being performed. Section 4 on post-

decontamination sampling design further explores the selection of the number of samples to be collected and their location with respect to various acceptance levels.

The organizations conducting the field sampling and laboratory analysis should be identified. If rapid response to a CW event is necessary, the only real analytical option is to have on-site analytical capability. This is especially true if decontamination teams are mobilized, and data is required to make determination of decontamination efficacy. If, however, it is possible to isolate and secure the contaminated area, and there exists no pressing need for the area to return to its intended use, it may be possible to use off-site laboratories. However, use of such laboratories will require additional chain-of-custody procedures, and the transport of samples to the laboratory may be complicated. Thus, when ever possible, the use of on-site analytical laboratories should be considered.

Regardless of whether onsite of offsite analytical capability is used, it is essential to use laboratories with experience, certification and/or accreditation to conduct CW agent analyses. There are international requirements for the accreditation of laboratories under the CWC (Rautio 1993). However, these requirements focus on accurately identifying the presence of the CW agent, with less rigour towards quantifying the amount present. In the case of decontamination verification, both presence and amount of the CW agent is important. Even so, laboratories and mobile units accredited under the CWC would be appropriate for use for decontamination verification. Other laboratories (such as those used or operated by the USDOD or FBI for CW agent analysis) could also be used. The laboratory should have documented procedures for the analysis of CW agents, a documented QA/QC program, and precision and accuracy data on the laboratory's CW agent procedures available for review. In addition, the laboratory should have the capacity to handle the potentially large number of samples generated from any sampling event.

2.2.1. Sample control and documentation

It is necessary to develop a method to document sample collection and maintain sample control. Sample control is conducted through the establishment and maintenance of a documented chain-of-custody.

It is essential to be able to determine the location of any sample with a positive result for additional decontamination. This may be especially problematic when many wipe samples are being collected from various surfaces. This issue is also discussed in Section 4. Once a sample identification scheme is selected, all subsequent sample documentation is based on the sample identification label. Existing guidance from the hazardous waste cleanup field details necessary sample tracking, documentation, and

chain-of-custody (USEPA 1997). Procedures have also been developed for sample tracking of CW samples collected as part of the CWC verification (Rautio, 1993). These include procedure GS 4 Coding and GS 6 transport log.

The existing guidance on sample tracking and documentation has in common the use of controlled logbooks to record field activities, the establishment and maintenance of chain of custody, and the use of a sample bottle label. Each bound logbook assigned to the sampling event is given a unique identifier. All logbooks used should be assigned such an identifier, and the list of logbooks carefully controlled. Entries into the logbook should reflect the sampling event as accurately as possible and include the date and time of sampling, scheme for selecting sample identifiers, subsequent sample identification of samples collected, the method of sample collection, condition of the site relevant to sample validity when applicable, results of associated field measurements (such as on-site meterological data) and calibration information pertaining to the field instruments used, and the name of the field personnel performing the work.

If the samples are being transported to an off-site laboratory, a formal chain-of-custody form should be filled out for each sample collected and follow the sample through transport, analysis, and final data reporting. Samples must be under the direct control of the individual signing for the samples on the COC form at all times. This includes storing the samples in a locked, secure facility under the control of the COC signatory. The COC record should specify the field logbook number which documents the sampling event. It should also include the analytical laboratory name, samplers name, project name, unique sample identification, the date and time of sample collection, the sample matrix and container, the required analysis and turn-around time and any additional instruction to the laboratory such as preservation requirements. The date and time the sample is relinquished and by whom and the date and time it is received by the carrier or analyst is noted on the COC. Sufficient carbon-copies of the COC should be available so that each signatory receives a copy. If the analysis is performed by an onsite laboratory, the COC can be abbreviated, and in some cases the logbook will suffice to establish COC.

Sample bottle identification should be clear and unambiguous. The sample label should include the project name, sample date and time, samplers name, preservation method if any, requested analysis, and any additional comments. Waterproof ink should be used. Ideally, a method to generate printed labels should be used to avoid errors in hand labeling.

2.2.2 Shipping samples

If offsite laboratories are used it will be necessary to ship the collected samples to the laboratory. Such shipping may result in a significant effort if the samples are considered equivalent to "neat" agent, indeed, it may be impossible to ship such samples (McGuire, 1992). There is significantly more latitude if the samples are considered environmental samples, which typically have low or negligible concentrations. This may be the case after decontamination procedures. There are recommended procedures for shipping samples collected under the CWC (Rautio 1993). These procedures make the distinction between samples of bulk product and potentially-highly contaminated materials (GS 2 Recommended operating procedure for packing of samples containing chemical agents) and environmental samples (GS 3 Recommended operating procedures for packing environmental samples). In the United States, samples are considered hazardous if they meet criteria as defined by 49 CFR part 171, Section 8. Even following these regulations, some common carriers may refuse to accept the samples. Thus, when considering the use of off-site laboratories, the method of sample shipment must be thoroughly explored.

2.3. Sample collection

2.3.1. Types of samples

The type of sample to be collected depends on the matrix to be sampled and the end use of the analytical result. Table 2.2 lists the primary types of samples which can be collected and analyzed for the presence of CW agents.

The collection of swipe samples typically involves use of a cotton swab wetted with an appropriate solvent (such as methylene chloride or acetylnitrile) which is used to swab a precise surface area (Rautio 1993). A typical wipe kit consists of two types and three sizes of forceps and a hemostat. The wipe material is ultra clean cotton, which will withstand slight scrubbing during collection. A wash bottle is filled with the solvent of choice and is used to moisten the wipe which assists in sample collection. A hemostat may be useful since it holds the wipe very securely which allows for vigorous scrubbing (McGuire, 1993). Once an area is sampled, the swab in stored in a clean glass vial for transport to the analytical laboratory, where the swab is extracted, and the extract analyzed for the presence of CW agent. Swab sampling is routinely used under the CWC to determine the presence of CW agents, and results can be used to evaluate contact hazard (Jenkens et al 1994). Large numbers of samples can be collected and analyzed in a reasonably short period of time. However, because the swab is not in contact with the sampled surface for more than a few seconds, significant extraction of sorbed CW agent from the surface being sampled into the swab is unlikely. Thus, this

method may not detect low concentration of sorbed CW agent that may still present an inhalation hazard. In addition, there does not currently exist information that would allow the determination of potential inhalation hazard from the results of surface samples. Still, because of its ease of collection and analysis, swab samples are probably the best current means for rapid determination of extent of contamination on surfaces and subsequent decontamination efficacy.

Table 2.2. Types of samples which can be analyzed for the presence of CW agents.

Sample Type	Advantages	Disadvantages
Swipe	– Rapid, easy sampling of surfaces – Large number of samples can be easily collected – reasonably easy chemical analysis – can provide information for contact hazard analysis	– Sorbed CW agent may not be readily detected – Information not currently available to translate results into inhalation hazard
Chip/bulk sample	– Able to detect presence of sorbed CW agent – Provides more definitive proof of presence or absence of CW agent	– Destructive analysis, required partial destruction of surface being sampled – Difficult extraction procedures with potential for multiple interference – Limited number of samples can be collected – Information not currently available to translate results into inhalation hazard
Environmental (water, soil, vegetation)	– Large number of samples easily collected – Able to detect presence of sorbed CW agent – Results can be used for contact and ingestion hazard analysis – Useful in delineating extent of contamination in outdoor scenarios	– Difficult extraction procedures with potential for multiple interference – Information not currently available to translate results into inhalation hazard
Air	– Ability to detect CW agents in a large, general area – Direct information on inhalation hazard – Some units can provide real-time results – can provide for long-term monitoring – can be used to monitor historical artifacts (artwork, documents, etc).	– May be more difficult to pinpoint precise areas requiring decontamination or re-decontamination – Results will not provide information on contact or ingestion hazards

Chip or bulk samples are actual pieces of the contaminated surface that are transported in a clean container (usually a glass jar) to the laboratory for analysis (Rautio 1993). The

sample is further ground and extracted with the appropriate solvent, with the extract analyzed for the presence of CW agent. This type of sampling allows for the detection of CW agent sorbed into the material, and thus can more definitively determine the presence of CW agent. However, there is a great deal of heterogeneity between samples due to the chemical composition of the material being sampled and the dispersive properties of the CW agent with the material (Jenkens et al. 1994, Rautio 1994). For example, it is well known that concrete, as an alkaline matrix, results in the rapid degradation of most chemical warfare agents. Thus, results from any single sample are less than reliable. In addition, like swipe sampling, it is currently not possible to translate the results of the analysis into an inhalation hazard. Its destructive nature also limits the number of samples that can be collected. In addition, the more complete extraction is more time consuming and difficult due to the possibility of co-extracting substances that could interfere with the chemical analysis, particularly . However, this method may be useful in the final steps of decontamination verification as more definitive proof of decontamination efficacy.

Water, soil and vegetation samples are actually a special class of bulk samples. These samples can usually be readily collected into clean glass containers in the field (Rautio 1993). The limiting factor here is not the number of samples that can be collected in the field, but the speed in which they can be analyzed in the laboratory. The analysis of the soil and vegetation samples is as laborious and difficult as the chip surface material samples, with the same potential for analytical interference. Water samples will be somewhat easier to analyze for the presence of CW agent. There are a variety of tools than can be used to collect environmental water samples, including syringes, vaccutaineers, Teflon tubing, bailers, etc. The choice of sampling equipment will depend of the environment in which the sample is being collected. Syringes may be most appropriate for small puddles, where as bailers or pumps with Teflon tubing best used for large ponds. For soil samples, surface deposition of the agent is the most likely course of contamination, thus sampling is conducted to obtain surface soil samples. A clean ruler is used to measure out an area of about six to ten centimeters square. If necessary, a clean spatula is used to score the surface to loosen the dirt to a depth of about 1 cm. A clean spoon is used to fill a clean glass bottle. It is important that the analytical laboratory sufficiently homogenize the sample if a subsample is to be analyzed. This is to ensure fractionation of soil particles based on size has not occurred, as differences in chemical attraction to soil particles of different sizes may effect subsequent analytical results.

Results of air sampling provide the most direct evidence of the presence of a CW agent, as most CW agents of concern (list here) have a significant vapor pressure. In addition, the air pathway is the pathway of highest concern with respect to human exposure. High-volume air samplers can sample over a large area. While this may be useful for

determining the presence of CW agent, it is less useful for determining the precise location of CW contamination to guide decontamination activities. Small, hand-held chemical agent monitors (CAMs), such as those used by the military (Harris and Shanty 1993), may be useful for rapidly monitoring smaller areas, although care would need to be exercised by sampling personnel to ensure all areas were sampled. And while the results of air monitoring can provide direct information on inhalation hazards, it does not provide information on contact or ingestion hazard. In the event that historical artifacts such as priceless artwork or documents have become contaminated with CW agent, these artifacts would be damaged by the use of chemical decontaminants. These artifacts could be placed into a closed container in which fresh air is vented through it, with the intent of flushing out the CW contamination. Exhausted air would be monitored for the presence of CW agent to determine when the artifacts are no longer contaminated. Finally, air monitoring could also useful for long-term monitoring of a building or facility after re-occupancy.

2.3.2. Sample collection methods

Table 2.3 summarizes the sample collection methods for the various sample types and sample matrices. A combination of sample types is likely to be the most effective for decontamination verification. For example, air sampling can provide information concerning overall effectiveness of decontamination. A positive detection in an air sample could be followed up with either wipe samples or chip/bulk samples.

Samples should be collected in the appropriate container that have been cleaned and silylated using approved protocols (such as the GT 5 protocol in Rautio 1993). Soil and water samples should be preserved with methylene chloride to inhibit biological activity. Most other samples can be preserved through freezing at –20 C.

Sampling must be conducted in such a manner as to prevent the cross-contamination of samples. This is important as positive results from the samples will require additional decontamination activities, thus cross-contamination of clean samples with dirty samples will result in unnecessary additional decontamination. Frequent changing of gloves, handling clean bottles from the outside only, and avoiding touching the insides of lids are all methods to reduce contamination. The CWC uses a hot person – warm person – cold person technique to prevent contamination (McGuire 1993). The hot person is the person actually collecting the sample. This person only comes into contact with the actual collection equipment (forceps in the case of wipe samples, spatula for soil samples, picking or chipping tool for other bulk samples). Collection equipment should be cleaned between each sample, and provided by the warm person. This can be accomplished by rinsing the tools in a solvent such as methylene chloride or iso-propyl alcohol. The warm person obtains a clean sample bottle from the cold person,

opens and holds the sample bottle for the hot person to fill, and closes the filled container. If wipe samples are being collected, the sample bottle contains the cotton swab and solvent, which the hot person extracts using clean forceps. The warm person labels the sample with the collection location. The warm person the places the filled sample container into a zip-lock bag held open by the cold person. The hot and warm person will generally wear full protective equipment. The cold person is responsible for sample tracking and documentation.

Table 2.3. Sampling methods by matrix for chemical warfare[a] agent decontamination verification.

Matrix	Material to remain *in situ?*	Sampling method	Sample Size	Sampling solvent	Preservation	Sample Container[f]
Air	yes	Hand-held CAMs[g]	variable	NA	NA	NA
		Low volume air sampling (SC1[e])	variable, up to 30 dm^3 at 20 C	NA	Store –20 C	3
Soil	yes	bulk sample (SC 5, SP 4[e])	50 g	NA	wet soil: 1 ml $MeCl_2$ Store –20 C	1
Water	maybe	bulk sample (SC 6, SP 7[e])	50 ml	NA	25 µl $MeCl_2$ Store –20 C	2
Vegetation	maybe	bulk sample	50 g	NA	Store –20 C	1
		Low volume air sampling (SC1[e])	Variable, up to 30 dm^3 at 20 C	NA	NA	3
Marble	yes	wipe (SP 5[e])	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Wood unvarnished	yes	wipe[b] (SP 5[e])	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Concrete/ plaster	yes	chip sample (SP 8[e])	50 g	NA	Store –20 C	1
		wipe[b]	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Asphalt	yes	chip sample	50 g	NA	Store –20 C	1
		wipe[b]	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Metal unpainted	yes	wipe[b] (SP 5[e])	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Floor tile	yes	wipe (SP 5[e])	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Paint/ rubber/ polymeric surfaces (includes paints & varnishes)	yes	chip sample (SP 9[e])	6 g[h]	NA	Store –20 C	1
		wipe[b]	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1

Table 2.3. cont.

Matrix	Material to remain *in situ?*	Sampling method	Sample Size	Sampling solvent	Preservation	Sample Container[f]
Painted dry wall	maybe	chip sample	50 g	NA	Store –20 C	1
		wipe[b]	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Ceiling tile	maybe	chip sample	50 g	NA	Store –20 C	1
		wipe[b]	4 in^2 cotton wipe	1:1 iso/$MeCl_2$[c]	Store –20 C	1
Carpet, tapestry, office partitions, linoleum, wallpaper	unlikely	bulk sample	50 g	NA	Store –20 C	1
Historic artifacts[d]	yes	Low volume air sampling (SC1[e])	Variable, up to 30 dm^3 at 20 C	NA	NA	3

a Includes the nerve (G and V) agents, the blister (H) agents, the blood agents (hydrogen cyanide, cyanogen chloride and arsine), and the choking agents (phosgene, chlorine diphosgene and chlorine).

b A rubber speta-extractor unit is under development and may be useful for this matrix.

c 1:1 isopropyl alcohol and methylene chloride.

d It is assumed that historic artifacts such as paintings, documents, etc, will not be treated. Agent residual should be monitored by sealing the artifact in a chamber and monitoring the chamber head-space for the presence of agent.

e Procedure number from Rautio 1993

f Sampling Containers:

(1) 250-ml silylated (GT 5[e]) wide-neck shatterproof glass jar with teflon or teflon-lined screw-caps .

(2) 50-ml silylated (GT 5[e]) shatterproof glass vial with teflon or teflon-lined screw-caps.

(3) Purified Tenax tubes and calibrated air pump

g Chemical Agent Monitor used by the Department of Defense. A hand-held sensor that can detect the presence of chemical warfare agents. It can discriminate between mustard and nerve agents and can respond within one minute (Harris and Shanty 1993).

h Mesilaakso and Tolppa 1996

Note: various solvents have been cited for use with wipes: methanol and isopropyl alcohol in McGuire 1993. Alcaraz (?) says "appropriate solvent, such as isopropyl alcohol, dichloromethane, etc". Rautio 1993 (SP 5) says use of alcoholic solvents may lead to esterification and trans-esterification. The solvent recommended above came from McGuire 2000. Thus, the sampler should confer with the chemical analyst to determine the most appropriate wipe solvent for use on a given matrix for a given agent.

2.3.3. Quality Control Samples

The collection and analysis of quality control samples should be done to provide information on the reliability of the sampling event. Both USEPA (USEPA 1997) and the CWC guidelines (Rautio 1993) provide guidance on the collection of QC samples. In general, QC samples consist of equipment rinsate blanks, matrix blanks and the

collection of co-located samples. Equipment rinsate blanks are taken by rinsing the collection tools (forceps, spatulas, bottles) with a solvent after decontamination and analyzing the rinsate for the presence of CW agent. Matrix blanks consist of an uncontaminated material of the type being analyzed. For example, if swipe samples are used, a matrix blank would consist of an unused swipe. Co-located samples (i.e. duplicate) samples should be collected from approximately 10% of all the locations sampled. However, because most CW agents are not found naturally in the environment or used industrially, it is unlikely that any of these chemicals would have a significant "background" signature. Thus, of these three major types of QC samples, the most important in the context of decontamination verification is the co-located samples. However, equipment and matrix blanks are useful in investigating matrix interference problems.

2.4. Chemical Agent Sampling References

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3. Collection and Processing of Microbiological Samples

3.1 Purpose

This section describes procedures for the microbiological examination of surfaces and air to determine the extent of contamination or the success of decontamination. Total viable microorganism counts can aid in evaluating the effectiveness of a decontamination material or procedure or monitor microorganism populations but will not determine which organisms are present. The methods presented are commonly used techniques however, the limitations of the procedures must be understood for an appropriate interpretation of the data. New technologies that require expensive equipment are not included.

3.2 Considerations for consistent microbiological sampling

3.2.1 Sampling Methodologies

Each determination consists of three phases: sample collection, sample processing, and the actual measurement. The manner of sample collection and sample processing can influence the outcome the measurements and so all three procedures must be taken into consideration when the results are interpreted. Microbial activity will correlate with numbers and biomass only as long as environmental conditions remain constant. A knowledge of background or pre-existing microorganism populations would aid in the interpretation of results. Any change in temperature, nutrient availability, or other environmental determinants may alter microbial activity or numbers, consequently, a careful record of the conditions in which the sample was taken is required.

When evaluating a site for biological weapon agents (BWA), certain decisions will need to be made by the sample collector to enhance the likelihood for obtaining samples containing the targets of interest. This type of sampling is called judgmental sampling (see section 4 for a through discussion of sampling procedures). Because it may not be possible to revisit an area to collect more samples, judgmental sampling, or locating the spots most likely to have measurable concentrations of the target analytes is important. When developing sampling protocols factors such as field variation, sampling method, transport, type and number need to be carefully considered (Table 3.1).

Table 3.1. Sampling Protocol Development.

Question	Step in Procedure
1. What kind of field variation is expected?	Inspect and characterize field
2. What type of sample will be used?	Select sample type: air, surfaces, chip
3. How many samples should be taken?	Define strategy related to study objective
4. How is sampling to be done?	Define collection methodology including prevention of cross-contamination
5. How are samples to be transported, processed, and analyzed?	Define processing and analysis methods

From van Elsas J. D. and K. Smalla 1997.

The method of obtaining samples is determined by the physical properties of the area being examined, by the expected abundance of the microorganisms and by the enumeration or measurement procedures to be performed. Some sampling approaches are listed in Table 3.2. Samples should be of sufficient quantity to permit quantification of low cell counts. Sampling procedures must be adjusted to the local circumstances of the environment under investigation. These adjustments can be addressed by determining the likely interference levels that may be experienced during sampling and adjust the sampling procedures accordingly. For instance, a large volume of diluent for samples suspected of high counts will improve cell count accuracy.

Table 3.2. Comparison of microbial sampling approaches for various substrates[1].

Environment	Access	Expected Number	Sampling devices	Sampling processing
Air	direct	low	filters or Settle plates	concentration on filters
Water	direct or remote	high or low	containers or filters	dilution or concentration
Soil	direct or remote	high or low	grab or corers	serial dilution
Surface-flat	direct	high or low	swabs, press plates, scraping or washing	dilution or concentration
Surface- textured	direct	high or low	press plates or swabs	dilution or concentration

[1]*From* Atlas and Bartha 1987. Table 7.1.

3.2.2 Identification of sampling units

Depending on the sample surface or type slightly different sampling methods should be employed. For example a dusty or dirty sample location may require greater dilution. If the study site is a building then standard methods such as swab tests or press plates are suitable. It is assumed that furniture, carpeting and drapes will be removed and destroyed from the contaminated building. Prior to identifying the sampling units a decision needs to be made whether a sampling grid will be used or will there be

targeted judgmental sampling (see section 4). A set number of samples should be taken from areas which might harbor contamination. Areas such as heating and cooling vents should be sampled thoroughly. Other areas of concern are regions which may on occasion be moist or harbor microbial nutrient sources. These may include countertops, kitchen and bathroom areas. A pre-screening of the building to understand the air circulation patterns of the building will aid in locating potential hot-spot locations for microorganism deposition.

3.3. Standardization of sampling procedures

3.3.1 Sample documentation

Sample documentation include three forms (1) the field logbook; (2) the chain of custody; and (3) the sample bottle label. These are necessary for sample control and completion of required documentation to ensure traceable and defensible sample results.

Careful documentation of the sample method and test parameters in a log book is an important facet of the project. This log book should be assigned a number which is to be recorded on the chain of custody record (COC). Entries in the sampling field logbooks should reflect the sampling event as accurately as possible and include the date and time of sampling, sample identification, method of sample collection, condition of sampling site relevant to sample validity when applicable, results of associated field measurements, calibration information pertaining to field instruments and the name of the field personnel performing the work.

Establishing a COC for each sample that will follow the sample from its initiation to its completed analysis is extremely important. The appropriate signatures in the COC form must be obtained to properly document transfer of samples to the analytical laboratory. The COC record should specify the field log book number which documents the facility, experiment or environment sampled. The COC record also includes the analytical laboratory name, samplers name, project name, unique sample identification, the sample date and time, the matrix and container, the analysis and turnaround time and any additional instruction to the laboratory such as filtration required or preservation. The date and time the sample is relinquished and by whom and the date and time it is received by the analysts or carrier is part of the COC record. This record includes four copies which are distributed to official data distribution personnel.

Sample bottle identification is critically important for the success of the project. Sample identification includes the project name, sample date and time, sampler name,

preservation method, requested analysis and comments. Waterproof ink is recommended so that the information will not be lost if the sample container gets wet. Ambient air temperature or environmental conditions can be recorded in the comments box.

3.3.2 Sample transport

A major concern in transporting biological samples is ensuring that the microorganisms remain viable and active without multiplication until the testing procedures have been performed. If counts are to reflect accurately the numbers of viable microorganisms present in the sample at the time of collection, then processing must be accomplished quickly because microorganisms reproduce rapidly in the collection vessels, yielding artificially elevated counts.

Samples should be protected from ultraviolet light, heating or freezing. When transit times are less than 6 hours the sample may be maintained at the original ambient temperature. If 6 hours is insufficient time for transit of samples, the general consensus is to lower the temperature to less than 10°C to restrict the amount of growth and deleterious interactions between the intrinsic species present in the sample. These samples should be processed within 24 hours of retrieval. Presterilized containers and materials must be using for all samples, and a trip blank should be handled in the same manner as collected samples. Appropriate steps should be taken to insure the safety of personnel handling the microbial samples (see section 3.3.5).

3.3.3 Standard microbiological sampling methods

There are three main methods of sampling: 1) contact plates or slides using appropriate media, 2) swabbing, 3) air sampling. The choice of method will depend upon the situation. In general, contact methods are best for clean surfaces but swabs for dirty ones because it is possible to dilute the swab washings. It is fundamental to the success of any investigation that the microbiologist is fully conversant with the problem and is prepared to go and look at the site and talk to the personnel concerned. A basic knowledge of microbiological techniques is required for these procedures.

Typical testing procedures include contact inoculation or swabbing. Contact inoculation for surfaces and solid samples by pressing an agar slide against the sample for about 5 seconds. Swabbing methods use a sterile moistened swab to transfer the sample from sites that are difficult to reach by rolling the swab on the agar surface to transfer the microbes onto the agar for cultivation. These methods require cell growth and require incubation for approximately 3 days at 37 °C.

Samples collected for microbiological analyses should be opened in a sterile laminar-flow biocabinet. All laboratory transfers and dilutions should be performed using aseptic techniques. All glassware, media and material used for microbiological testing must be autoclaved at 121°C for 15 min.

Agar contact for flat surfaces

Agar filled contact plates and agar-covered slides are available from several companies. Some dip slides may be used as contact slides. It is possible to estimate total numbers, and major classifications, i.e. enterobacteria, yeast and fungi using an agar contact method. Some commercially available contact plates include a hinged plastic slide covered on both sides with an agar medium enclosed in an aseptic vial. These sampling kits are easy to transport to the test site and back to the laboratory. Slide may be inoculated by the contact method using a moistened sterile swab in sites that are otherwise difficult to reach, they can be dipped into liquid for aqueous testing.

Surface swab counts

It is generally accepted that the swabbing technique gives a count approximately ten times higher that that obtained by agar surface contact when sampling smooth surfaces. Typically, a 5 cm x 5 cm template is placed on the surface to be examined and the area is swabbed with a cotton wool or alginate swab. A swab is dipped in sterile 0.1% peptone water and rubbed over the surface to be tested. One swab is used for each predetermined area. The swab is returned to the tube containing a volume of 0.1% peptone. The tube containing the sample is mixed and allowed to stand for 20-30 minutes. The sample is diluted and plated using the appropriate media. The count/25cm^2 is given by the number of colonies/mL of rinsate or solvent multiplied by 10.

Air sampling

Settle plates supplement surface sampling for assessing potential surface contamination. Several plates containing appropriate media are exposed for a given time and incubated. Settle plates are commonly used to monitor air over long periods and to monitor hospital cross-infection. Several commercial air samplers are available, some samplers estimate air-borne microbes by counting the particulate matter. The particulate air count, while rapid and useful, does not replace a count of the viable airborne microorganisms. Common methods for checking this include exposure of Petri dishes containing nutrient agar for a given length of time, or a slit-sampling machine (a device that draws a quantity of air and impinges it on a revolving Petri dish containing nutrient agar, Sykes 1965). Sampling the air itself is best for assessing the load of smaller particles.

Method Control samples and background counts

Method controls are run with similar manipulations as the surface or air samples. A good practice is to use 5% of the total number of samples are method blanks (1:20 samples). Background microbiological counts should be established for the local vicinity by sampling a non-contaminated room of similar size and exposure. Sterility control are necessary to check the sterility of medium and dilution water blanks by pouring control plates for each series of samples. Additional controls to determine contamination of plates, pipettes and room air should be part of the analysis protocol. To insure quality of data, the analyst needs to demonstrate competence.

3.3.3.1 Colony Counting Criteria

The nutrient plates can be counted manually or by an automated counter. All plates of 300 or fewer colonies are counted. Plate samples should be diluted to give from 30 to 300 colonies/plate. The aim is to have at least one dilution giving colony counts between these limits. If the total number of colonies is less than 30 from undiluted sample, the 30 to 300 rule is disregarded and the results 0 to 30 are recorded according to Standard Methods (1989). With this exception, only plates having 30 to 300 colonies should be considered in determining the plate count. When the total number of colonies developing from 1.0 mL is less than 30, 2.0 mL may be plated however, no more than 2.0 mL is recommended.

The bacterial count per milliliter are computed by multiplying average number of colonies per plate by the reciprocal of the dilution used. The results are reported as colony-forming units (cfu) per milliliter. If plates from all dilution of any sample have no colonies, the count is reported as less than one (<1) times the reciprocal of the corresponding lowest dilution. If the number of colonies per plate exceed 300, the results reported follow the rules for estimation cited in heterotrophic plate count (9215) of the Standard Methods.

3.3.4 Biological weapon agents

The presence of increased total viable counts of microorganisms does not imply a BWA event. Isolation, purification, growth and amplification, and species (biotype) identification are necessary to determine if an agent has been released or is still present after decontamination activities. Total viable microorganism counts can aid in evaluating the effectiveness of a decontamination material or procedure or monitoring microorganism populations but will not determine which organisms are present.

Table 3.3 is a list from the Center for Disease Control of microorganisms that are potential biological weapon agents (BWA). Table 3.4 provides information on selected

BWAs and sampling methods. Species and biotype identification requires qualified laboratory personnel, specific techniques and laboratories equipped with biosafety devices.

3.5 Safety Equipment

This section does not purport to address all of the safety concerns. It is the responsibility of the user of the information to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

The safety equipment guidelines listed under Biological Safety Level (BSL) 2 and BSL 3 should be reviewed and incorporated as appropriate into protocols for work with unknown microorganisms. The CDC recommends the following guidelines in *Biosafety in Microbiological and Biomedical Laboratories*.

1. When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered.
2. Eye protection should be worn during sampling and when analyzing samples if an open-fronted containment system is used.
3. Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. For example, use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.
4. When handling dry forms of toxins that are electrostatic:
    a. Do not wear gloves (such as latex) that help to generate static electricity
    b. Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.
5. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.
6. Consider both toxin and diluent when selecting gloves and other protective clothing.

7. If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

Table 3.3. Center for Disease Control's list of restricted microorganisms.

Bacterial Agents	**Fungal Agents**
Bacillus anthracis	*Blastomyces dermatitidis*
Bordetella pertussis	*Coccidioides immitis*
Brucella (B. abortus, B. canis, B.melitensis, B. suis)	Cryptococcus neoformans
Burkholderia pseudomallei (Pseudomonas pseudomallei)	*Histoplasma capsulatum*
Campylobacter (C. jejuni/C. coli, C. fetus subsp. fetus)	*Sporothrix schenckii*
Chlamydia psittaci, C. pneumoniae, C. trachomatis	Pathogenic Members of the Genera *Epidermophyton Microsporum,* and *Trichophyton*
Clostridium botulinum Clostridium tetani	Miscellaneous Molds
Corynebacterium diphtheriae	**Viral Agents (other than arboviruses)**
Escherichia coli (Cytotoxin-producing (VTEC/SLT) organism*s)*	Hantaviruses
Francisella tularensis	Hendra and Hendra-like Viruses (includes virus formerly known as Equine Morbillivirus
Helicobacter pylori	Hepatitis A Virus, Hepatitis E Virus
Leptospira interrogans – all serovars	Hepatitis B Virus, Hepatitis C Virus (formerly known as nonA nonB Virus),
Listeria monocytogenes	Hepatitis D Virus
Legionella pneumophila; other *Legionella*-like agents	Herpesvirus simiae (Cercopithecine herpesvirus [CHV-1], B-virus)
Mycobacterium leprae	Human Herpesviruses
Mycobacterium *spp.other than* M. tuberculosis, M. bovis *or* M. leprae	Influenza
Mycobacterium tuberculosis, M. bovis	Lymphocytic Choriomeningitis Virus
Neisseria gonorrhoeae	Poliovirus
Neisseria meningitidis	Poxviruses
Salmonella - all serotypes except *typhi*	Rabies Virus
Salmonella typhi	Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV)
Shigella spp.	Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru and related agents
Treponema pallidum	Vesicular Stomatitis Virus
Vibrionic enteritis (Vibrio cholerae, V. para-haemolyticus)	
Yersinia pestis	

From: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Edition, Center for Disease Control

Table 3.4. Standard sampling methods for selected CDC restricted biological agents.

Bacteria	Description	Potential Sampling method(s)	Potential Initial Culture media	Potential Identification Method(s)
Bacillus anthracis[1]	Bergey's Group 18,rod-shaped and straight, 0.5-2.5 × 1.2- 10 um, often arranged in pairs or chains with rounded or squared ends. Motile by peritrichous flagella. Endospores are oval only one spore per cell, aerobic or facultatively anaerobic, Gram-positive	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent	Brain heart infusion agar containing polymyxin, lysozyme, EDTA, thallous acetate or glucose tryptone agar (yellow halo-acid production) or Difco nutrient agar	Identify B. anthracis by immunofluorescence; sera is commercially available – use 5% sodium hypochlorite solution 15 min exposure to kill cells – Polymerase chain reaction (PRC) unique primers
Brucella abortus Brucella melitensis Brucella suis[1]	Bergey's Group 4, aerobic, Gram-negative coccobacilli or short-rods 0.5-0.7 × 0.6--1.5 µm, intracellular parasites	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent	LKV enriched selective meduim for the isolation and partial identification	Polymerase chain reaction (PRC) unique primers
Burkholderia (pseudomonas) mallei Burkholderia (pseudomonas) pseudomallei	Bergey's Group 4 gram-negative rods 0.5-1.0 × 1.5 - 5.0 µm, aerobic	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent		Polymerase chain reaction (PRC) unique primers
Clostridium botulinum[1]	Bergey's Group 18 endospore-forming gram-positive rod 0.3-2.0 × 1.5-20.0 µm, obligately anaerobic	Swab applicator and media tubes or, diluent added to biological activity reaction tests or, RODAC in anaerobic chamber	C. difficile presumptive identification cycloserine-cefoxition fructose agar	Polymerase chain reaction (PRC) unique primers -– toxin inactivated by 0.1% sodium hypochlorite or 0.1 N sodium hydroxide
Francisella tularensis	Bergey's Group 4, obligately aerobic, Gram-negative rods 0.2 × 0.2-0.7 µm, produces H_2S agent in tularemia or rabbit fever, spread by ticks, deer fly and mosquito gram-negative bacillus, aerobe 0.2-0.7 um not transmitted from person to person	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent	grows at 37C media containing glucose and cysteine with blood.	Polymerase chain reaction (PRC) unique primers
Psaterurella tularensis [1]	Bergey's Group 5, small oval rods, gram-negative bacilli with capsules, 0.3-1.0 × 1-2 µm, non-motile, facultatively anaerobic	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent		Polymerase chain reaction (PRC) unique primers
E. coli 0157:H7	Bergey's Group 5, straight rods 1.1-1.5 µm, gram-negative, facultatively anaerobic	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent	Difco 9100-32-0 for E. coli 0157.	Polymerase chain reaction (PRC) unique primers

Table 3.4. cont.

Bacteria	Description	Potential Sampling method(s)	Potential Initial Culture media	Potential Identification Method(s)
Yersinia pestis	Bergey's Group 5, in risk/hazard group 3, small oval gram-negative bacilli with capsules, 0.5-0.8 × 1-3 µm, non-motile, facultatively anaerobic	Surface tests: Press plates, or RODAC plates Suspension tests: swab sampling into diluent	Yersinia selective agar base with cefsulodin-Novobiocin, selective enrichement for the isolation of Y. enterocolitica	Polymerase chain reaction (PRC) unique primers - IS100 insertial sequence chromosonal distribution analysis
Coxiella burnetii	Bergey's Group 9, rods or coccoid, gram-negative, lack flagella, obligate intracellular association	will not grow on bacteriological media require specialized cultivation techniques		
Rickettsia prowazekii Rickettsia rickettsii	Bergey's Group 9, rods or coccoid, gram-negative, lack flagella, obligate intracellular association	will not grow on bacteriological media require specialized cultivation techniques		

[1]Biological warfare agent

3.4. Validation and Verification of data generated by analytical laboratories

Validation and verification of data is necessary to ensure consistent results of a known quality so the data user can evaluate and make judgments based on the analytical results. The process begins with a review of the signed COC form for each sample received to determine if the COC had been broken. The condition of the sample upon receipt should be evaluated to determine if the samples were damaged or compromised during shipment. Samples should arrive at the laboratory at the proper preservation temperature and within the proper holding time. At least one method blank should be analyzed in every analytical batch of samples.

Detection limits are the smallest amount that can be detected above the noise in a procedure and within a stated confidence limit. Microbiological counts range from <10 up to 10^9 cfu/mL. Counts in this range may have large numeric deviations and depending on the dilutions used during processing, the results may have a large detection level value and therefore, microorganisms may be present but not in sufficient quantity to be quantified by spread-plate, most probable number (MPN), press-plate or other common viable plate counting methods. The level of detection should always be included with each result.

3.5. Microbiological Sampling References

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4. Post-decontamination Verification Sampling

4.1 Purpose

The purpose of post-decontamination sampling is to decide whether or not the decontamination process has successfully reduced contamination to a level below a "maximum acceptable level."

If a site were to be measured exhaustively then it might be possible to make a decision with 100% confidence of being correct. If not, the decision will unavoidably have some uncertainty. Exhaustive sampling would require, minimally, sampling every square inch of a site, and analyzing every sample with no errors by the analytical laboratory, no lost samples, no data transcription errors, and so on. Swiping an entire 30,000 square foot office building with four square inch swipes would require over 1 million swipes for the floors alone. Since exhaustive sampling is not possible, decision uncertainty is unavoidable.

Statistically designed sampling provides a quantitative estimate of decision uncertainty—provided that the necessary statistical design information is available.

This section is appropriate to discrete point sampling (in contrast to a scanning method). It does not address sampling methodology (e.g., swipes for a smooth surface vs. soil cores for a grassy field), but assumes that appropriate methods will be used. Sections 2 and 3 outline available methods for the collection of chemical and biological agent samples.

This section addresses the question of how many samples to collect and where. Two aspects are discussed:

1. Information and criteria necessary for a statistical design
2. Sampling logistics necessary to identify areas within which a statistical design can be used.

4.2 Literature Review

There is a substantial body of literature regarding post-cleanup verification in the context of environmental remediation and facility decommissioning from the US EPA, NRC, and DOE. We also reviewed some literature in the general area of infection control and environmental control in medical settings.

In the medical setting, there is substantial guidance on when, where, how, and how often to clean ("decontaminate"). There is little or no guidance on how to verify that cleaning procedures have been effective. For example, the CDC Guide on Handwashing and Environmental Control (Garner and Favero, 1985) states:

> Before 1970, regularly scheduled culturing of the air and environmental surfaces such as floors, walls, and tabletops was widely practiced in U.S. hospitals. By 1970, CDC and the American Hospital Association were advocating that hospitals discontinue routine environmental culturing, since rates of nosocomial infection had not been related to levels of general microbial contamination of air or environmental surfaces, and meaningful standards for permissible levels of microbial contamination of environmental surfaces did not exist (1,2). Between 1970 and 1975, 25% of U.S. hospitals reduced the extent of such routine environmental culturing (3), and this trend has continued.
>
> In the last several years, there has also been a trend toward reducing routine microbiologic sampling for quality control purposes. In 1982, CDC recommended that the disinfection process for respiratory therapy equipment should not be monitored by routine microbiologic sampling (4). Moreover, the recommendation for microbiologic sampling of infant formulas prepared in the hospital has been removed from this Guideline, since there is no epidemiologic evidence to show that such quality control testing influences the infection rate in hospitals.

The CDC guidance does indicate sampling when problems arise:

> Microbiologic sampling is indicated during investigation of infection problems if environmental reservoirs are implicated epidemiologically in disease transmission. It is important, however, that such culturing be based on epidemiologic data and follow a written plan that specifies the objects to be sampled and the actions to be taken based on culture results.

The environmental literature, in contrast, contains a great deal of guidance regarding post-decontamination or post-remediation verification sampling. Probably the premiere such document is the Multi-Agency Radiation Survey and Site Investigation Manual (MARSSIM), developed jointly by the US EPA, DOE, NRC, and DOD and issued in 1997 (EPA 1997).

The purpose of MARSSIM is to provide "detailed guidance for planning, implementing, and evaluating environmental and facility radiological surveys conducted to demonstrate compliance with a dose- or risk- based regulation" (page Roadmap-1). MARSSIM guidance "focuses on the demonstration of compliance during the final status survey following … remedial actions." MARSSIM includes guidance on quality assurance and quality control.

MARSSIM appears to be gaining widespread acceptance as a primary guidance document. For example, the US NRC describes MARSSIM as "containing acceptable methods for final status surveys" (NRC 1998). MARSSIM training is being offered nation-wide to the environmental remediation community on an on-going basis, and it is not unusual for regulatory agencies to ask for a MARSSIM approach to environmental investigations.

Although MARSSIM is intended for radiological contamination, the sampling design and QA/QC information is applicable to non-radiological contamination. The MARSSIM chapters that discuss measurement methods and instruments (primarily Chapters 6 and 7 and Appendices H and J) are not applicable to directly applicable to CBW, but most of the remainder of MARSSIM is useful and relevant. In fact, MARSSIM could probably serve as the primary guidance document for post-decontamination verification sampling following a chemical or biological warfare or terrorist event.

MARSSIM recommends rather simple statistical methods, presumably in the hope that they will be adequate in most cases, but does leave room for the use of more sophisticated methods if they are needed. One approach to verification sampling not discussed in MARSSIM, and therefore discussed more extensively here, is sampling to find areas that were overlooked by the decontamination process, or so-called "hot spots."

Another statistical approach not found in MARSSIM but possibly of interest for CBW is that of examining the upper percentiles of the contaminant distribution. This approach is discussed in EPA (1996) and Gilbert and LeGore (1996).

We expect that in the event of an actual CBW event, the criteria for deciding that a site has been adequately cleaned will be more stringent than envisaged by the authors of MARSSIM and other guidance documents. Therefore, this document includes additional discussion of the statistical concepts that must be understood in order to achieve the high level of confidence likely to be desired by stakeholders and the public.

Finally, should a CBW incident actually occur, the decontamination and sampling efforts will almost certainly be performed under a great deal more time pressure, public scrutiny, and health risk than expected in any of the guidance documents we have

examined. For this reason, we initiate a discussion of the challenges that will need to be met and logistical problems that will have to be solved (see Sections 4.4 and 4.6).

4.3 Inputs to statistical design

As mention in Section 4.1, exhaustive sampling is not possible and decision uncertainty is unavoidable. Therefore, statistically designed sampling is essential.

In order to design a sampling plan statistically the "maximum acceptable level" must have a quantitative definition. This is a level above which the site is not clean enough. We anticipate that criteria for a maximum acceptable level will be defined in one or more of the following ways:

- By specifying a largest acceptable "hot spot."
- By specifying a maximum acceptable average concentration.
- By specifying a maximum acceptable value for a specified concentration percentile (for example, "90% of the surface should have fewer than 10 spores per square meter").

The environmental literature generally recommends either random sampling or sampling on a grid when designing decontamination verification sampling.

In addition to statistically designed sampling, we anticipate that some judgmental sampling will take place. For example, locations expected to have higher concentrations due to airflow patterns would probably be sampled in addition to any statistically designed sampling. Table 4-1 summarizes the three decision criteria and three sampling designs discussed in this section.

Table4-1. Summary of sampling designs and situations

	Sampling design		
Criteria	**Grid**	**Random**	**Judgmental**
Hot spot	Sections 4.3.1 and 4.5.1	Not applicable	May take place
Average	Sections 4.3.2 and 4.5.2	Sections 4.3.2 and 4.5.2	Not applicable
Percentile	Sections 4.3.3 and 4.5.3	Sections 4.3.3 and 4.5.3	Not applicable

It must be strongly emphasized that the sampling approach, statistical design, and data evaluation depend on the nature of the risk and the exposure pathway. For example, if dermal contact to even a small spot of residual contamination may have a serious effect than hot spot statistics are probably best. On the other hand, if inhalation of

contamination out-gassing from a surface is the primary hazard, and it is believed that averaging takes place due to mixing in the air, then statistics based on the average concentration are probably most appropriate.

In this document, we attempt to anticipate several possible risk and exposure scenarios and suggest sampling design approaches appropriate for each.

4.3.1 Inputs to statistical design: hot spots

The concept of a hot spot is simple and intuitive: a relatively small contaminated area within a generally clean area. The concept is rather broad. For example, hot spots can have many different sizes and shapes. Issues include questions such as how much greater than the surrounding area should the level of contamination within the hot spot be in order to call it a "hot spot" and whether the level of contamination within the hot spot is relatively uniform or varies widely?

This document defines a hot spot similarly to a number of regulatory guidance documents and other publications (EPA 1996, Gilbert 1987, Gilbert and LeGore 1996), as an area that is:

- Contiguous,
- Shaped roughly circularly or elliptically, and
- Contaminated in its entirety above a maximum acceptable level.

The shape assumption is necessary in order to develop a mathematical model for the probability of detection. Since hot spots can in reality have a variety of shapes, the calculated probabilities are approximate.

In order to design a sampling plan to address hot spots, these criteria must be specified:

- The size, and possibly orientation, of the hot spot,
- The maximum acceptable level, and
- The desired probability with which a single hot spot must be discovered.

In order to design a sampling plan the size and contaminant level of a largest acceptable hot spot, and the probability with which a single such hot spots must be detected must be set.

4.3.2 Inputs to statistical design: averages

We include the average as a potential basis for decision criteria because it is suggested in the environmental literature. For example, EPA 1996 states, "The mean is of course well-known as an excellent estimator of long-term, chronic phenomena, where long-term exposure is based on averaging the effects of many possible exposure events over a period of time."

In order to design a sampling plan to address the average concentration on a surface two contaminant levels must be specified:

- The maximum acceptable average level, and
- A "detectably clean" average level, below the maximum acceptable level.

The detectably clean level is a level below which it is highly desirable that the sampling effort will correctly conclude that decontamination was successful. In principle, any average level below the maximum level is acceptable. However, an average level just slightly below the maximum acceptable level is practically impossible to distinguish from the maximum acceptable level—in other words, not detectably clean. Hence the need to specify a detectably clean level.

In order to design a sampling plan acceptable chances of incorrect decisions must be specified. Two types of decision error can occur:

- An unsuccessful decontamination may be declared successful (a false positive or "false clean").
- A successful decontamination may be declared unsuccessful (a false negative or "false dirty").

Below the detectably clean level the false negative decision error rate should be acceptably small. Above the maximum acceptable level the false positive decision error rate should be acceptably small. The range of concentrations between the detectably clean level and the maximum acceptable level is sometimes called the "gray region" (EPA 1997). Within the gray region, false negative error probabilities become relatively large. It is not practical to conduct a sampling effort in which the detectably clean level equals the maximum acceptable level; there must be some separation between them.

The maximum acceptable level, the detectably clean level, and the acceptable decision error probabilities must *all* be specified prior to designing and performing a post-decontamination sampling effort. Hopefully they will have been set prior to any actual real-world event.

In addition, the number of samples necessary to achieve the specified error rates depends on the variability of contaminant concentrations after decontamination. This variability depends on the nature of the contaminant, how the contaminant was dispersed, the nature of the contaminated media, the decontamination methodology, and how well the decontamination was performed. It can not be assumed the variability will be the same in all cases. However, it should be possible to estimate a range of possible values from experiments used to test the efficacy of various decontamination methods. Without such information it is not possible to statistically design a sampling effort that controls (in advance) the decision error probabilities.

Of the two decision errors described above, the second will result in additional expenses due to unnecessary additional decontamination and delay before the site can be returned to productive use. The first error, however, is more serious, because it puts people at risk. The statistical sampling design takes this into account by using the following decision making process:

- Assume the sampling unit is still dirty (the conservative assumption and the statistical null hypothesis).
- Collect data using design based on acceptance and decision error rate criteria.
- Reject the assumption only when the data *strongly* counters the assumption.

This is the "dirty until proven clean" approach, which will result in stronger evidence than the "clean until proven dirty" approach. In a post-decontamination situation, the "clean until proven dirty" approach is equivalent to being overconfident in the effectiveness of the decontamination process.

4.3.3 Inputs to statistical design: percentiles

We include the estimation of upper percentiles as a potential basis for decision criteria because, as described in EPA 1996, "…in some situations there may be greater interest in possible acute effects or transient exposures associated with significant short-term risk. Such exposure events may not happen often or on a regular basis…." This is similar to the exposure model of the hot spot approach, except that with hot spots the higher levels are assumed to be concentrated in small distinct areas, whereas with percentiles they are considered to be scattered throughout the sampling unit.

In order to design a sampling plan to address percentiles, two percentiles of the post-cleanup contaminant distribution must be specified:

- The percentile associated with the maximum acceptable level
- A "detectably clean" percentile.

Suppose, for example, the maximum acceptable level is 10 units. The decision criteria might be that the 90th percentile of the contaminant distribution must be below 10 units. The detectably clean percentile might be 99%, meaning that if the true 99th percentile is in fact 10 or below then it is highly desirable to correctly conclude the cleanup was adequate.

In order to design a sampling plan acceptable chances of incorrect decisions must be specified. Two types of decision error can occur:

An unsuccessful decontamination may be declared successful (a false positive or "false clean").

A successful decontamination may be declared unsuccessful (a false negative or "false dirty").

4.4 Sampling design overview

Many factors other than statistical design influence the success of a sampling effort. This section introduces a discussion of some of these issues, and describes a potential approach to sampling logistics.

4.4.1 Sampling logistics

Before using a statistically based sampling design, it is necessary to specify clearly and unambiguously the area within which the sampling design will be used. In complex sites for which this document is intended, this is a non-trivial issue.

Conceptually, the approach is the same for different kinds of sites—enclosed (buildings), semi-enclosed (stadia), open (plazas, parks) and mixtures (airports). The discussion in the next section refers to buildings.

The essence of this approach suggested here is to systematically divide a site into small practically sized sampling units and then collect samples from each sampling unit using a sampling design appropriate to the decision criteria and the nature of the unit. A large complex site is broken down into (many) small and manageable sampling units, each of which is sampled with a simple sampling design. Different kinds of sites will have different kinds of sampling units.

With this approach, a separate decision is made for each sampling unit. A decision about an entire site will be made based on the collective decisions for the (many) sampling units.

Careful, detailed, and thorough record keeping will be essential to assuring that the verification sampling is thorough.

4.4.2 Buildings

For each building, the following steps are necessary.

- Systematically identify, tabulate, and label each and every room that was decontaminated (the term "room" is used to represent any distinct space, including entryways, hallways, lobbies, closets, etc.).
  - — Within each room identify, tabulate, and label all distinct surfaces and objects that need verification sampling.
    - – Surfaces include walls, ceilings, floors, countertops, windows, etc. (door surfaces will be considered as part of the wall in which the door is set).
    - – Objects include doorknobs, handrails, sinks, faucets, etc.
- When the tabulation is complete the samplers should have:
  - — A complete list of every grid-able surface in the building. These are the sampling units.
  - — A complete list of non-grid-able objects that should be considered for subjective sampling (for example, handrails and doorknobs).
- For each sampling unit:
  - — Identify an "origin" for the grid. Samplers will measure distances to sampling points from this origin.
  - — For vertical surfaces the lower-left corner is the origin (pre-defined by this procedure).
  - — Non-vertical surfaces can have their origin defined by the samplers for convenience, provided, of course, that the origin is recorded.
  - — Starting from the origin and the grid spacing identify the sampling locations.

- A location-naming scheme needs to be developed. Verification documentation should be coordinated with decontamination documentation.
- A system for marking sample locations may be necessary (for example, in order to return to locations where residual contamination exists).
- Collect samples.
- Pack samples in transportation container, deliver to analytical service, etc.
- Review analytical results, make decisions, repeat decontamination where indicated

4.5 Sampling designs

This section provides additional discussion of the statistical criteria for sampling for hot spots, averages, and percentiles.

4.5.1 Sampling for hot spots

This section describes details of designing a sampling grid for sampling units where acceptance criteria are based on the presence or absence of hot spots.

Grid spacing is calculated using a probability model developed by Singer (1972, 1975) of the U.S. Geological Survey for locating geologic deposits. The method is also described in EPA 1996, Gilbert (1987), and Zirshky and Gilbert (1984).

The probability model exists for three types of grids: square, rectangular, or triangular. Of these, triangular appears to be the most efficient, but it is also the most difficult to use. We anticipate that decontamination teams will be under a great deal of pressure in the field and believe that the simplest grid, the square grid, should be used.

The probability of finding a target hot spot using grid sampling depends on the hot spot size and the grid spacing. Or, given a hot spot size and a desired detection probability, the necessary grid size can be calculated. Figure 4-1 illustrates a sampling grid that has an approximately 90% probability of detecting an 11 inch diameter hot spot on an 8 foot by 12 foot surface. On a larger surface, the grid spacing would remain the same, and more samples would be collected.

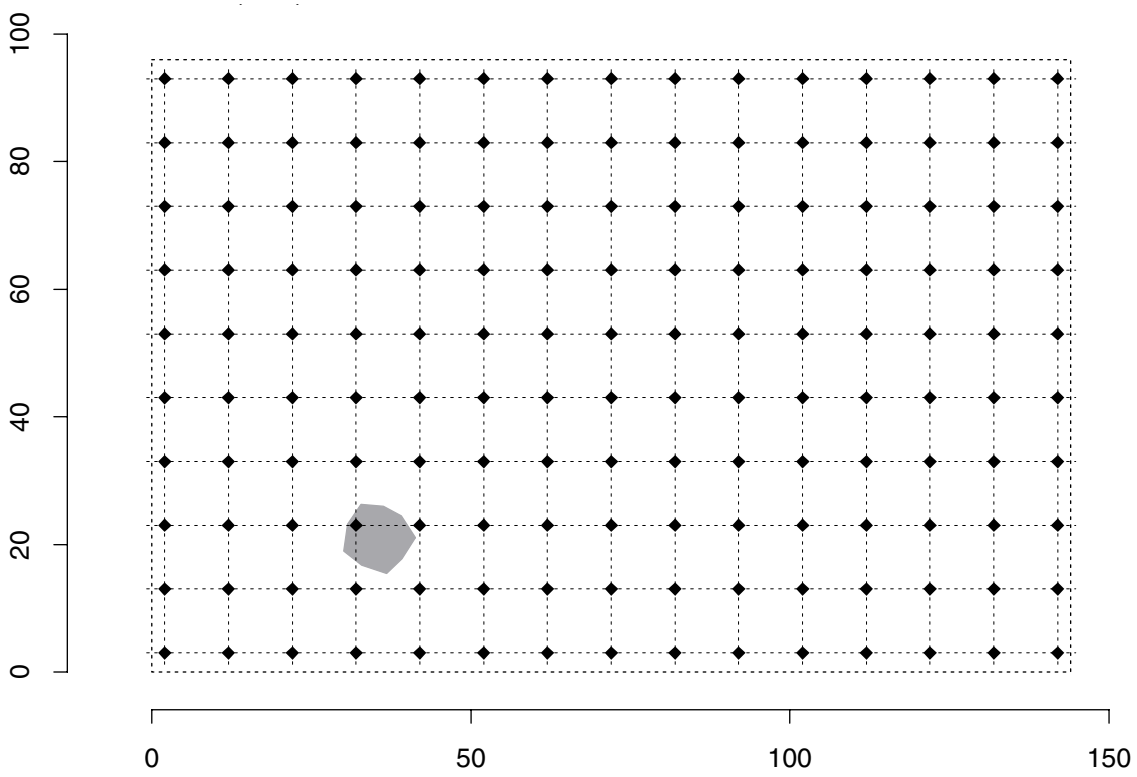

Figure 0-1. Example sampling grid with 10-inch spacing on an 8x12-foot wall. The hot spot diameter is approximately 11 inches; hot spots of this size have an approximately 90% probability of being found. The origin of the grid is 2 inches in and 3 inches up from the lower left corner.

Table 4-2 lists grid intervals in inches that achieve each of several combinations of hot spot size and desired confidence level (probability of detection). The total number of samples depends on the size of the sampling unit surface.

Table 4-2. Grid spacing (in inches) necessary to achieve specified detection probabilities for various hot spot sizes.

Hot spot Size		Confidence Level (Detection Probability)				
Area	Radius	50%	80%	90%	95%	99%
4 square inch	1.1	2.8	2.2	2	1.9	1.7
36 square inch	3.4	8.5	6.7	6.1	5.7	5.1
1 square foot	6.8	17	13	12	11	10
4 square feet	13.5	34	27	24	23	21
1 square meters	22.2	56	44	40	37	34

As an extreme example, consider a sampling effort that requires a 99% probability of finding a 6 inch diameter hot spot on a single 8 foot by 12 foot interior wall using 2 inch by 2 inch sample swipes. As shown in Table 4-3, this would require 2,400 swipes and result in swiping about 69% of the wall surface. Since this is a single wall in a single room, it should be clear that requiring such a large confidence for such a small hot spot in a site such as a large building would be totally impractical.

Table 4-3. Number of samples necessary to find a hot spot on a single 8x12-foot interior wall, using 2x2-inch sample swipes.

	90% Confidence		99% Confidence	
Hot spot diameter (inches)	**Number of samples**	**Percent of wall swiped**	**Number of samples**	**Percent of wall swiped**
6	1734	50	2400	69
8	672	19	925	27
10	345	10	486	14
12	216	6.2	294	8.5
18	88	2.5	117	3.4
24	48	1.4	54	1.6
30	24	0.7	35	1
36	20	0.6	24	0.7
42	15	0.4	20	0.6
48	12	0.3	15	0.4

Figure 0.2 illustrates the relationship between confidence level, hot spot diameter, and number of samples.

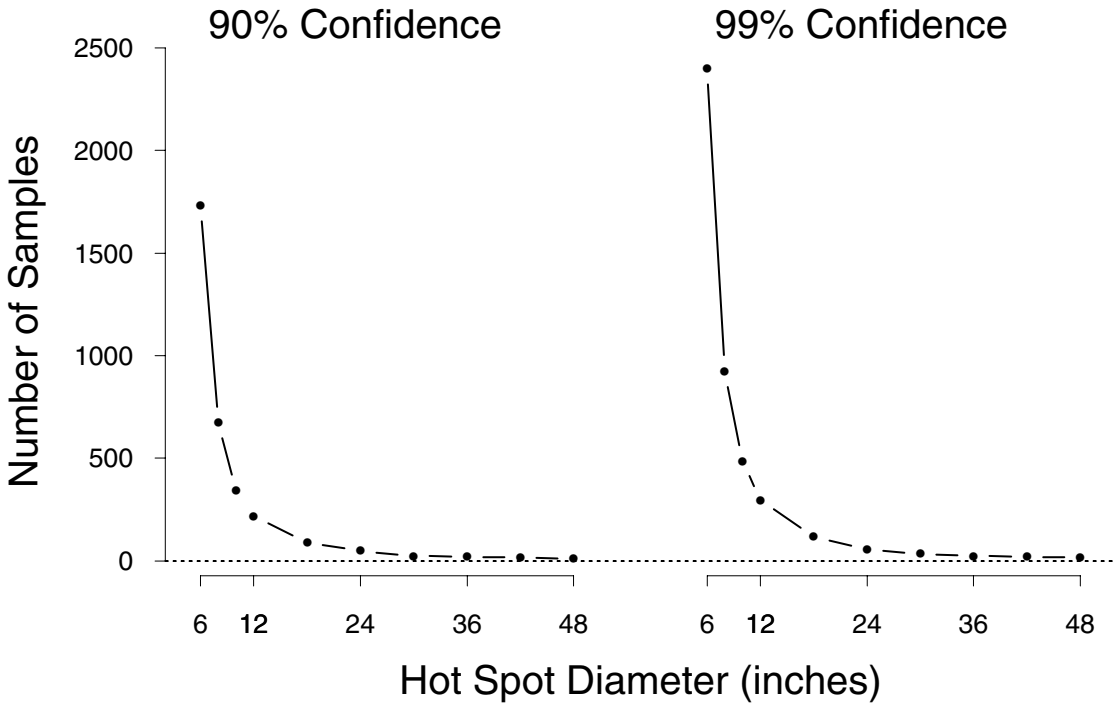

Figure 0.2. Number of samples required as a function of hot spot diameter.

The probability model uses some simplifying assumptions. These are:

- The hot spot has a circular or elliptical shape.
- Elliptical hot spots have a random orientation.
- The sample size is small relative to the hot spot size.
- Detection is certain to occur if a sampling location (grid point) falls inside a hot spot.

Of course, no hot spot will ever be exactly circular. In the absence of information or experience indicating what size or orientation of ellipse would be a better approximation, Table 4-2 was calculated using a circular hot spot. It would be valuable to develop such information during the decontamination-testing phase of this project. In particular, the force of gravity on vertical surfaces may lead to tall narrowly shaped residual contamination, in which case a rectangular grid will probably be better.

More extensive tables of grid spacings can be calculated when that becomes necessary.

4.5.2 Sampling for averages

The statistical theory used to determine the number of samples that have to be collected is discussed in many statistics books and regulatory guidance documents (Bury, 1975, EPA 1996, Hardin and Gilbert 1993, Snedecor and Cochran, 1967).

Suppose, for example, that the maximum acceptable level is set at 20 units and the detectably clean level is set at 15 units. Then the size of the gray region is 20 – 15 = 5 units.

The goal is to collect enough samples to reliably detect a difference of 5 units, given a specified level of protection against false clean (false positive) decisions.

Suppose that the standard deviation of the residual contamination is 12 units. Then n = 91 samples must be collected in each sampling unit in order to have a 95% chance of correctly concluding that the cleanup criteria have been met (if they have), with a 1% chance of incorrectly concluding the criteria have been met (if in fact they have not).

In this example the relative standard deviation is 12/5 = 2.4. Table 4-4 shows the number of samples as a function of the relative variability. The false positive (false clean) rate was set to 0.1%, a very protective level, and sample sizes are calculated for each of three false negative (false dirty) rates.

Table 4-4. Number of samples required when sampling for averages when sampling with a 0.1% chance of incorrectly concluding the decontamination criteria have been met.

	False positive rate = 0.1% False negative rate		
Relative standard deviation	**10%**	**5%**	**1%**
0.5	7	8	10
0.75	15	17	21
1	26	29	37
1.25	40	45	58
1.5	57	65	83
1.75	77	89	112
2	101	116	147
2.25	127	146	186
2.5	157	180	229
2.75	190	218	277
3	226	259	329
3.25	265	304	387
3.5	307	353	448
3.75	352	405	514
4	401	461	585

Note that when the number of samples is largest when the variability is largest. After acceptable levels have been established contaminant variability is the most crucial factor for determining the total number of samples required (and thus the cost and duration of the verification sampling). If sufficient time is available, it would be possible to perform the verification sampling in two phases. Phase one would be a small effort of perhaps 30 to 100 samples for the purpose of estimating contaminant variability, and the second phase would be a full sampling effort, with the number of samples calculated from the variability measured in the first phase. Such an approach would naturally require that the portion of the site selected for phase one would be representative of the entire site.

4.5.3 Sampling for percentiles

Table 4-5 shows the number of samples required for several combinations of criteria. For example, if the criteria are that

- 90% of the contamination be below the acceptable level,
- the false positive (false clean) rate should 0.1% (very protective), and

- the false negative (false dirty) rate should be 5% when 95% of the contamination is below the acceptable level (i.e., when the sampling unit is cleaner than required), then 662 samples need to be collected.

Table 4-5. Number of samples required when sampling for percentiles.

		False positive rate = 0.1% False negative rate		
Acceptable percentile	**Detectably clean percentile**	**10%**	**5%**	**1%**
90%	95%	583	662	823
95%	99%	402	439	512
95%	99.9%	213	220	233
99%	99.9%	1495	1596	1793
99.9%	99.99%	15072	16079	18056

The statistical theory underlying these calculations is found in a variety of sources, including Hardin and Gilbert 1993.

4.6 Open issues:

4.6.1 Risk policy

Many sampling design decisions depend on the acceptance criteria that are in turn based on risk assessments. Therefore, the criteria need to be set by appropriate policy makers before any "final" or near-final sampling plans can be developed.

Criteria should be as specific as possible. For example, different types of sampling units (floors, walls, ceilings, doors) may have different acceptance criteria. Interior walls might use different criteria (hot spot) than grassy fields (average). Criteria may be different for different contaminants. Hopefully, most or all decisions of this will have been made prior to an incident.

Hopefully, the criteria and sampling design will be known prior to planning for an actual incident.

4.6.2 Logistics, documentation

This document outlines an approach to for an actual decontamination effort. A much more detailed procedure that is practical and effective for field use needs to be developed and tested. Field decontamination teams must be involved in its development.

During this sampling event decontamination has not yet been verified. Therefore, the work is likely to be done with the same level of PPE as the decontamination team used. This in turn implies, for example, that record keeping will be difficult (it will not be practical to carry around a clipboard and take notes, fill out field tracking forms, etc.). There are an immense number of practical details related to sample handling and sample tracking that need to be worked out and tested.

It is possible that multiple sampling teams will enter the site, either from the same entrance or multiple entrances. The record keeping system needs to accommodate this. This means, for example, that simply numbering the rooms incrementally as they are encountered will not work. Existing room names or numbers could be used, but they will not always be available or practical, so a general algorithm likely to work for any site should be developed and tested. Possibly, the distinct rooms may already have been identified, tabulated, and named during the decontamination effort. If so, these might be used.

In order to assure a complete tabulation of sampling units, some sort of systematic approach must be developed. For example, part of this procedure could be to always proceed counter-clockwise around a room from its entrance point.

Should there be two passes through a site: one to identify and tabulate sampling units, and a second to actually collect samples? Or should samples be collected as sampling units are identified?

The naming scheme needs to be easy for the samplers to use. It should allow more than one individual to collect samples from the same surface at the same time without getting confused. The scheme should make it easy to return to and decontaminate any residual contamination that is found.

It is assumed that moveable objects such as paintings, potted plants, desks, chairs, telephones, filing cabinets, water coolers, etc. will have been removed and disposed of; only fixed and integral structures remain.

Many moveable objects are large, heavy, and difficult to move. This is likely to substantially increase the risk to personnel involved in the decontamination. For example, should such objects be removed and disposed of prior to decontamination? This may present serious health risks, both to the movers and during transportation and disposal. On the other hand, if they are left in place, they may interfere significantly with the decontamination effort, and indeed, may in and of themselves be difficult or impossible to decontaminate (e.g., a sofa, large potted plant, microwave oven that happened to be open).

Since moveable objects have been removed the number of sampling units has been reduced as much as possible.

Since we assume that moveable objects have been removed, we also assume that the entire extent of surfaces will be accessible.

A related issue is what kinds of sampling design tools need to be developed? Is there a need for a software package running on a portable computer that can be used in the field to calculate grid spacing? This envisages a scenario where a sampling team is working inside a site and communicating by radio to support staff outside who would run the calculations and send instructions to the team. Something of this sort will probably be necessary for sample tracking, in any case.

Will grid spacing be calculated in the field during a decontamination effort, or will they have been pre-calculated before an incident occurs? As an extreme, will grid spacing be calculated separately for each surface as it is encountered, or will the sampling teams enter a site already knowing the spacing for every surface or every type of surface?

4.6.3 Statistics

The statistical "multiple comparisons" issue needs to be considered. That is, if there are several hundred sampling units in a large site, and each individual decision is made with, say, a 1% false positive probability, then the likelihood of there being at least one false positive decision among them all is quite large.

Discussions are for surfaces. Non-surface objects need some other method that is to be determined. Curved surfaces may also use grid-based or random sampling if the curvature is not too great.

It is anticipated that sampling of (smooth) surfaces will be done primarily by swipes. Such an intensive sampling effort does not lend itself to obtaining chipped or bulk samples of the surface itself, which is often the preferred sampling method for chemical agents (see Sections 2 and 3). Each swipe is a 2-inch by 2-inch square. If it turns out, based on risk and exposure criteria, that even very small areas of residual contamination are unacceptable, then the sample size may not be small relative to the hot spot, which would violate one of the model assumptions. Until the risk/exposure criteria and policies have been established it is unknown whether an effort to correct the model for violations of this assumption would be worthwhile.

A 2-inch by 2-inch swipe may only partially overlap an area of residual contamination. Depending on the degree of overlap, detection may not occur. Also, if a hot spot does

not have a well-defined boundary, but gradually tapers off, sampling in the taper region may not detect the hot spot.

Probability formulas for grid sampling assume the sample size (swipe area) is small relative to the sampling unit area and small relative to the hot spot size. An approximate adjustment to the probability calculation has been developed for situations when these are not the case.

The handling of non-detections has not been discussed. It is discussed in some of the references that have been cited.

The sample size calculations for the average assume that the number of samples is large enough that the statistical test can use the normal distribution, rather than the small-sample t-distribution.

There is an alternative statistical method for percentiles, based on tolerance intervals, that may allow fewer samples, provided that the residual contamination follows a normal distribution. We are unwilling to make this assumption at this time, so we have not presented the other method. However, it remains in consideration.

4.7 Glossary

Approval criteria	Conditions that a sampling unit must meet in order to be approved for return to normal use.
Decision error	Either of two incorrect decisions: (1) deciding a sampling unit is sufficiently clean when in fact it is not, or (2) deciding a sampling unit is not sufficiently clean when it fact it is.
Detectably clean level	A level that is considered sufficiently clean, and for which it is highly desirable that sampling unit will correctly be declared clean.
Detection probability	The probability of detecting residual contamination as large or larger than the maximum hot spot size.
Distribution	With reference to residual contamination, the range of levels, and the proportions of the sampling unit that remain contaminated at various levels within that range.
False clean	Deciding a sampling unit is sufficiently clean when in fact it is not. A synonym for false positive.
False dirty	Deciding a sampling unit is not sufficiently clean when in fact it is. A synonym for false negative.
False negative	Deciding a sampling unit is not sufficiently clean when in fact it is.
False positive	Deciding a sampling unit is sufficiently clean when in fact it is not.
Gray region	The range of contaminant concentrations between the detectably clean level and the maximum acceptable level
Grid spacing	The distance between grid-based verifications sampling locations. For a square grid, the spacing is the same in both directions.
Hot spot	A relatively small contaminated area within a generally clean area.
Judgmental sampling	Subjective selection of sample locations by an individual. Preferably based on expert knowledge of the process being studied.

4.7 Glossary cont.

Maximum acceptable level	The highest level that may be considered sufficiently clean. Above this level it is highly desirable that the sampling unit will correctly be declared not clean.
Null hypothesis	A statistical term that refers to the default assumption made at the beginning of a decision-making process.
Percentile	A value below which a specified percent of a population lies.
Random sampling	Selection of sample locations so that every potential sampling location has an equal chance of being selected.
Residual contamination	Contamination left behind by a decontamination process.
Sampling unit	Portion of a site or facility considered as one unit for the purpose of verification sampling.

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5. Summary of Open Issues: Areas for future effort

This document summarizes existing procedures for the collection of samples for analysis of chemical and biological warfare agents in order to verify decontamination efficacy. We have also outlined the necessary steps required in order to develop a statistically-based sampling procedure. In compiling this information, we have identified several areas requiring additional work before we can claim readiness in the event post-decontamination verification is necessary.

The most pressing open issue is the need for specific acceptance or cleanup criteria in a form that can be used to generate a statistically-based sampling plan and with which to compare subsequent analytical results for the purpose of determining verification efficacy. These criteria should be in the form appropriate for the type of sampling being conducted. For chemical weapon agents, appropriate units would be mass/unit area for swipe samples, mass/mass for bulk samples, and mass/unit volume of air for air samples. For biological weapon agents, criteria should be in the form of cfu/unit area for surface samples, cfu/mass for bulk and environmental samples, and cfu/volume of air for air samples. For these criteria to be meaningful, they should relate to an actual human health hazard, below which such hazard would not be expected. For example, an acceptance criteria for a chemical agent on surfaces in mass/unit area could relate to either a contact hazard or an inhalation hazard resulting from expected volatilization from the surface. It should be clear whether the acceptance criteria applies to individual sample results or an aggregate average of results.

Besides the obvious need for development of cleanup criteria, several other areas require additional effort. We recommend that a general quality assurance project plan be prepared for decontamination verification. The QAPP would list requirements for laboratories conducting CBW analysis. This would include the type of analytical methods the laboratories should conduct, documented method development and validation, specific criteria with respect to spike recoveries, duplicate precision, and other quality control criteria. These laboratories should have well documented safety procedures and maintain the capability of handing live agent (both CW and BW).

Either as a component of the QAPP or as a separate document, a list of all fixed and mobile laboratories meeting the requirements for conducting CBW analysis should be compiled and carefully controlled to ensure it remains current. In addition, detailed sample transportation protocols should be developed and approved should the use of fixed, offsite laboratories be necessary.

In addition to the general QAPP, specific QAPPs should be developed for each of the major scenarios which lay out specific acceptance criteria for each scenario. It is anticipated that

acceptance criteria may differ between scenarios based on the degree of accepted risk. Development of scenario specific QAPPs obviously cannot occur until progress is made on the development of health-based cleanup criteria.

There are also several issues still outstanding with respect to biological organisms. Specific protocols for confirmatory identification of a viable organism as a BW agent should be developed. As already mentioned in Section 3, standard plate counting techniques are not sufficient to positively identify the species of microorganism under culture. We recognize there is currently a great deal of research and development being conducted with respect to the use of immunoassay and PCR techniques for the detection of BW agents. These techniques will need to standardized for use with either standard plate counting or other viability-determining technique should positive identification of the presence of remaining BW agents be desired after decontamination activities. Standard plate counting techniques can be used to provide initial information concerning remaining viable organisms after decontamination activities. It should also be pointed out that sampling for viruses and biotoxins have not been addressed in this document.

As discussed in detail in Section 4, several specific sampling and statistical technicalities need to be addressed in more detail. A detailed sample naming and tracking protocol should be developed to allow the tracking and management of potentially hundreds of samples. A decision logic should be developed to allow selection of the most appropriate type of sample to be collected. With respect to statistical design, should an actual event requiring decontamination occur, it will be natural for everyone involved to want to be "highly confident" that the cleanup has been sufficient. The challenge is to translate "highly confident" from the subjective to the quantitative. Subjective decisions will be very vulnerable to second-guessing. Quantitative decisions less so. The example sample size calculations in Section 4 have been chosen, deliberately, to illustrate the fact that when "highly confident" is translated into, for example, a very large probability of detecting a very small hot spot (Table 4.3), the resulting number of samples is extremely large. Inevitably, high confidence comes at a high cost, and quite possibly at a higher cost than has heretofore been anticipated.

We believe most of not all of these items should be in place prior to the need for post-decontamination verification. This would provide solid, defensible data to provide to stakeholders

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