Sampling for Biological Agents in the Environment
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4 The Role of Sampling in the Phases of a Biological Event: Fact and Fiction in an Airport Scenario

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Where no plan is laid, where the disposal of time is surrendered merely to the chance of incident, chaos will soon reign.

VICTOR HUGO

Biological events can be divided into three distinct environmental sampling phases: initial assessment, characterization, and clearance. This chapter examines the different sampling goals and strategies associated with each phase to provide an overall perspective and framework for understanding the role of environmental sampling in a biological event. Specific sampling methods are mentioned; however, this chapter is not intended to provide a comprehensive review of either sampling or analysis methods. Such detail can be found in chapters 5, 6, and 9. A fictional airport scenario is provided in order to better convey the sampling issues and to place them in a realistic context.

Coordination during a Response

No single entity possesses the authority, resources, and expertise to unilaterally handle an incident from start to finish; hence, coordination and communication are critical to successfully manage a biological event. Coordination and handoff issues are briefly discussed during each phase, since different sets of investigators, each with unique roles, may be involved in an event.

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Initial Assessment: Is Contamination Present?

Initial assessment takes place in the first 24 to 48 h after the discovery of an incident. The atmosphere during this time is tense, characterized by rapidly unfolding information and ever-looming deadlines. There are three basic incident discovery scenarios: discovery of physical evidence (e.g., a suspicious package); detection of an agent by an environmental sampling surveillance system network such as BioWatch or other such networks, which are discussed further in chapters 6 and 9 (3); or reports of medical symptoms or disease, typically occurring some time after the incident and based on suspicions that the illnesses are not the result of a natural disease outbreak. The airport scenario which follows is based on the discovery of physical evidence.

Fictional airport scenario

It is a sunny Tuesday morning at the Megalopolis International Airport. A businessman who has just arrived on the red-eye from the coast is waiting for his bag at the luggage carousel when he notices that another bag on the carousel appears to be giving off a small, steady white cloud of dust (Fig. 1). He yells out for security or a police officer. Within 5 min, the carousel has stopped and the police have evacuated the area. The police attempt to retain witnesses in an adjacent area for questioning, asking people not to leave or touch their luggage. Interviews with the waiting passengers suggest that about one-quarter of the passengers had already collected their luggage and left the area before the suspicious bag was noticed and police were called to the scene. First responders begin arriving, and additional responders and experts are called for further evaluation.

Figure 1. Suspicious white-powder incident at an airport baggage claim area. The illustration combines the initial observed event and the later step of cordoning off the area around the baggage carousel.

What if you were involved in this response? Where should environmental samples be collected to evaluate whether contamination is present? Who might have been exposed in the immediate area? While this area is the top priority, should you be concerned about other areas if results come back positive? Who else might have been exposed? What if the device was generating aerosol prior to arriving at the baggage carousel? What about the workers who offload the baggage? What testing could be done to check this? Is contamination likely to have been spread by those who claimed their bags and left before the suspicious bag was noticed?

Coordination issues during the initial response

The initial assessment phase may involve sequential sampling from the different groups involved in the response and investigation. The following discussion identifies some of the these groups and clarifies their roles.

First responders

Local emergency responder or hazardous-materials (hazmat) teams may be the first ones called after facility occupants discover a suspicious package, such as the suitcase at the Megalopolis International Airport. Their objective on arrival is to assess the situation, take steps to stabilize conditions, and evacuate persons in the area to a safer location. While most reports turn out to be hoaxes or false alarms, any indication that an incident might represent terrorism involving a biological agent triggers the need to notify the Federal Bureau of Investigation (FBI) Weapons of Mass Destruction Operations Unit (4). The FBI leads the law enforcement effort to assess whether an incident poses a credible threat and notifies the Department of Homeland Security, which in turn informs other agencies. In cases deemed to be credible, first responders typically create exclusion zones around the areas considered most likely to be contaminated and decontaminate evacuated individuals.

First responders may use handheld assay devices to sample suspect powder materials (Fig. 2). These are presumptive tests, used to screen materials deemed suspicious to determine if they are chemical, biological, or radiological. Although further on-site biological screening tests are available, they have important limitations (refer to chapters 5, 6, and 9 for more information on these devices). The Centers for Disease Control and Prevention (CDC) and the General Services Administration have cautioned against reliance on these methods, given their low sensitivity and their potential to produce both false-positive and false-negative results (2, 7).

Law enforcement

The primary law enforcement sampling objective is to identify and confirm if a biological agent is present and, if so, locate the source of the contamination to aid the criminal investigation. Local FBI personnel typically take over the location from first responders once occupants are removed from

Figure 2. Handheld assays are antibody-based tests that are often employed in the field to give presumptive information about a sample.

the area. The location is classified as a crime scene, and access is restricted to law enforcement personnel in order to ensure that forensic evidence can be collected without compromise. Decisions on where to perform additional tests are made by the FBI in coordination with the Department of Health and Human Services (4). Given our airport scenario, the samples that were collected would be quickly transported to a Laboratory Response Network (LRN) facility, where cultures are to be performed and preliminary results are made available within 12 to 24 h. FBI activities may also include interviewing occupants, examining visual evidence, and working to reconstruct the sequence of events. If possible, agents collect the delivery source (letter, device, suitcase, etc.) and any additional "forensic" environmental samples from the source and adjacent surfaces to help piece together what occurred and who might have been involved. Sampling methods employed by law enforcement include surface samples using press plate, swab, wipe, or vacuum methods (chapter 5 contains further discussion of various surface sampling methods).

Public health

Public health environmental sampling objectives include conclusively determining that contamination is present and quickly identifying the population at risk. For example, while forensic sampling might determine that a device or piece of evidence is contaminated, public health investigators are interested in knowing about the contamination in the room where the samples were collected, including all nearby spaces and high-traffic areas. Is the contamination localized to the baggage carousel, or has it spread to the taxi stand or the food court? What is the occupancy of those areas? Is there evidence that the biological materials were aerosolized?

Public health sampling explores the spread of contamination as it relates to the potential exposure of employees, occupants, or the public. The investigators aim to define the areas of contamination and potential exposure via environmental sampling combined with engineering input and epidemiological findings (12). For example, finding surface contamination on the tops of air ducts or rafters that were unlikely to have had contact with a contaminated source or finding a dispersion pattern of multiple positive results might suggest that aerosolization has occurred during or after an event (1). Aerosolization in turn suggests that inhalation exposure may have occurred for any employees in the area at the time of the event. This can affect risks. For example, inhalation anthrax is a life-threatening illness and is more serious than cutaneous anthrax. The cutaneous form of anthrax is the main risk in cases where aerosolization is limited but surface-to-surface contamination nonetheless leads to skin exposure. Secondary aerosols can result from disruption of settled surface particles. Studies of simulated simple activities such as handling of contaminated papers or foot traffic on contaminated floors resulted in secondary aerosolization of viable *Bacillus anthracis* spores in a study done in a contaminated U.S. Senate office (16).

Public health investigators gain access to the site either through coordination with law enforcement personnel or after law enforcement investigators have completed their sampling and lifted crime scene restrictions. Law enforcement personnel do share sampling data and relevant incident details with public health officials, although some data may be deemed law enforcement sensitive. Data sharing is important because initial public health decisions, such as whether to administer "postexposure prophylactic" antibiotics and other medical countermeasures, need to be made quickly after potential exposure to be most effective. For example, estimates suggest that in anthrax, for each day that postexposure prophylaxis is delayed after an aerosol exposure, the case-fatality rate can increase by 5 to 10% (10).

Where to sample? Developing a public health sampling strategy

Because sampling every surface within a building or contaminated area is not practical, a strategy is required to select surfaces for sampling. Details of surface sampling are discussed in chapter 5 and are briefly described for this scenario. Targeted sampling, sometimes also called judgmental sampling, is often the primary strategy used (6). This means that investigator judgment is used to determine which surface locations have the greatest chance of being contaminated so that they can be evaluated first. Depending on circumstances, it may be obvious to investigators where to sample (e.g., the floor area where a device was found), but if no source is visible it may not be obvious where to begin. Targeted sampling is the most expeditious way to find contamination, although it is only as good as the information on which it is based. The following steps describe a framework for developing a strategy to identify the locations most likely to have been contaminated.

Step 1: Evaluate the Circumstances of the Event via Interview and Observation

Observation input

Investigators should don protective equipment and visually inspect the affected area to note physical details. This allows an opportunity to examine heating, ventilation, and air-conditioning (HVAC) equipment, machinery, and other relevant features and enhances the understanding of details provided from interview information.

Interview input

Investigators need to gather all available information about the reported event and timeline from law enforcement personnel and others who were on the scene. Examples of questions include the following.

Questions for law enforcement officials and first responders

- What is known about the likely delivery source and agent?
- How much time elapsed between the estimated time of the initial release and discovery of the evidence?
- Has the source been recovered? If so, does it appear to have been in the same place the entire time or might it have moved along a certain route prior to discovery?
- Do forensic tests provide any clues about the characteristics of the contaminant (e.g., particle size, spore concentration, presence of additives)?
- Were affected employees decontaminated after the event? What was the evacuation route by which they left the area?
- Was any affected equipment removed for forensic sampling? If so, what was the evacuation route by which it was removed from the area?

Questions for workers and managers at a facility

- What tasks or operations took place between the time the incident is thought to have taken place and the time it was discovered? For example, did any maintenance or custodial activities occur?
- How many employees were likely to have been in the incident area or to have passed through it?
- Did the source move along a route? Was it moved as part of a work process?
- Were machines involved with the process? (This is important because some machines, such as mail-sorting equipment, can aerosolize contamination, which creates the potential for inhalation exposure and the spread of contamination.)

- Was the HVAC system shut down after the incident was reported? If yes, how long did it operate prior to shutdown? Was it a total or partial shutdown?

Interviews with managers should include those familiar with the facility ventilation system. In addition, it is useful to view and obtain copies of facility ventilation plans.

Questions for physicians, epidemiologists, and other public health personnel

As noted at the beginning of this chapter, incidents may be triggered not by the discovery of physical evidence but by reports of symptoms or disease. The 2001 anthrax-related bioterrorism events were initially triggered by reports of anthrax found in individuals. Public health investigators were involved from the beginning and worked with law enforcement colleagues to interview patients and coworkers in order to "trace back" to potential exposure sources. These clues were successfully used to identify sampling locations. Examples of questions include the following:

- What is known about the person's activities during the incubation time frame? What is known about the person's job?
- What exposure circumstances does the job suggest?
- What clues do these details provide about potential release points? About timing of releases?
- What hobbies or interests does the person have?

Step 2: Identify All Plausible Contamination Pathways

Interview and observation information is combined in this step to form hypotheses about plausible contamination pathways. The term "pathway" is used to describe mechanisms and routes for contamination spread from surface to surface, surface to air, air to air, and air to surface. Knowledge of pathways is related to investigator expertise, experience, and familiarity with published reports of previous episodes. Potential pathways also vary depending on the biological agent—the key difference being that some agents, such as smallpox virus, are contagious and can involve a person-to-person pathway whereas other agents, such as *B. anthracis* spores, do not.

Process pathways

Process pathways are pathways associated with a work activity or sequence of steps or movements along a given route. Processes may be either manual or mechanical. For example, letters sorted at a postal facility follow a specific pathway involving several types of automated sorting machines. The machines use belts to move the letters, and this action can expel and aerosolize the contents of the letter. Recovery of the "source" letters in 2001 allowed investigators to identify the specific machine mail path via the sorting codes stamped on the letters during the cancellation process (5). This information was used for targeted sampling of those machines.

In an airport setting, baggage handling can be viewed as an example of a process pathway. Checked luggage pieces follow a defined process pathway outbound from customer to screening to conveyors to handling carts to the plane and then back again along a route to the final luggage carousel at the destination airport. Carry-on luggage takes a different pathway. In a case where a piece of checked baggage is involved in a terrorism episode, the path taken (specific conveyors and carousels) by the recovered piece of luggage would represent a plausible pathway for surface sampling considerations.

Foot traffic pathway

Biological materials can be spread from surface to surface or into the air by individuals who step on the bioagent or whose clothing becomes contaminated with bioagent particles; the extent of the spread is dependent on the persistence of the bioagent in the environment. For this reason, interviews with employees should include questions about the original routes traveled to and from the incident area, since these routes represent plausible pathways. For example, in the case of the Hart Senate Office Building and the letter mailed to Senator Tom Daschle, contaminated floor locations were found to reflect footprints of staff from nearby offices who entered Senator Daschle's suite to see what was happening. In addition to these primary routes, secondary routes taken by employees when evacuating the building with first responders also represent potential contamination pathways, as do routes taken by responders or law enforcement personnel in removing evidence for further testing. Again, in the case of the Hart Senate Office Building, responders led employees from the contaminated sixth floor offices to the ninth floor of the building for additional testing (9). Surface contamination was later found along this route. Surface contamination was also found in vehicles, equipment, and surfaces in the office suite used by the emergency responders (11). Investigators looking for foot traffic pathways should also consider carts or other such equipment, since wheels can create the same type of surface-to-surface spread of agents as footwear.

Air movement pathways

Moving air also represents an important contamination pathway for spreading airborne materials, and it is important to consider return air registers and ducts closest to the site of the incident. Because particulates in the air are attracted to nearby electrostatically charged surfaces such as televisions and computer screens, these can also be considered part of the air movement pathway. Once particles are suspended in the air, they enter the ventilation system and may be pulled throughout the system. Most commercial building ventilation systems recirculate air during heating and cooling seasons, which can affect the likelihood of spread of aerosols throughout a building. Some offices use floor level units to recirculate air from the office, and these locations should also be considered. Ventilation parameters, such as the amount of outside air being received, design features, and ventilation filter efficiency ratings, all affect the likelihood of spread. It is important to check the first prefilter and filter on the return air side of the HVAC fan unit for air movement pathways. As might be expected, contamination was found at these locations at the Hart Senate Office Building.

Maintenance and other activity pathways

Investigators should ask whether any maintenance activities occurred between the estimated time of the incident and discovery. If so, it is important to learn what was done and what methods were used to discern whether any activities could have aerosolized contaminants. At the Brentwood (now the Curseen-Morris) postal processing and distribution center, compressed air was used to clean the sorting machines at the end of each shift. This process is thought to have been a contributing factor in spreading the contamination through the facility (5). Similarly, because most vacuum cleaners are not HEPA vacuums, their filtration systems may not capture microscopic organisms. Instead, they re-entrain surface contamination into the air and raise the risk of inhalation exposure for nearby occupants and the custodial workers operating the equipment. Brooms and mops can also spread contamination, not only at the incident site but also to areas that are cleaned subsequently, such as adjacent offices.

The "other" pathways through which biological agents can spread need to be identified through an epidemiological investigation that takes into account the unique properties of the agent. For example, anthrax is not known to be communicable (spread from person to person) other than by inadvertently transporting spores on clothing or other similar physical means; however, person-to-person spread may be an important route for other biological agents.

Step 3: Develop a Sampling Plan To Target the Locations for Each Plausible Pathway That Are Most Likely To Be Contaminated

Once plausible contamination pathways have been systematically considered and inventoried, investigators can use this information to develop a sampling plan. The sampling plan should evaluate each plausible contamination pathway. Specific locations can then be targeted based on where the best available evidence suggests that positive results will be obtained. For example, in a case where foot traffic is a plausible pathway for the presence of an agent inside a room, the doorway is the most likely to be contaminated since all occupants must pass through that point. All such locations should be carefully described and documented.

Composite samples: how can they help?

Composite samples can optimize initial sampling by maximizing the surface area that is included in a sample. This can be done by taking several wipe samples and combining their results or using HEPA sock methods over a larger surface area. Composite sampling can be particularly helpful if minimal information is available for identifying plausible pathways or if sampling or analysis resources are limited. The disadvantage is that a positive sample can be traced back to the entire composite area but not the specific area of each swipe, thus diminishing the resolution of the sample.

What about air sampling?

While air sampling provides more relevant information for potential inhalation risks, it has drawbacks for initial assessment use. First, because a positive air sample result does not identify the surface that is contaminated, surface sampling is still necessary. Second, time delays between the incident and the sampling may result in airborne contaminants either moving or settling onto a surface depending on variables such as size of the particle, ventilation, and occupancy conditions. Thus, negative air sample results are not sufficient by themselves to allow a conclusion that contamination is not present in detectable amounts; surface samples are also necessary. Positive air sampling results can serve as a warning by suggesting the possibility of continued exposure via reaerosolization. In summary, air sampling can be used during initial assessment, but surface sampling is the primary sampling tool. Further details about air sampling can be found in chapter 6.

Evaluation of results

If results are negative, how sure are you that contamination is not present?

If all results come back negative, investigators must use caution before concluding that contamination is not present in detectable amounts. Targeted sampling is only as valid as the information on which it is based. Gaps in details of the incident introduce uncertainty, raising the possibility that available information was insufficient to identify pathways or the locations most likely to be contaminated. Thus, it is important that investigators not leap to conclusions simply because the first round of sampling produces negative results. Such conclusions may be reliable in cases where incident details are clear, but in other cases it is recommended that investigators take additional steps and perform supplemental sampling before drawing conclusions. While this chapter focuses on sampling strategy, it is also important to remember that limitations in current sampling methods should be taken into consideration. Method validation and information on sensitivity, accuracy, and lower limits of detection are still being developed for available sampling methods.

It is also important to understand that while environmental sampling information is valuable, it is not the only source of information and should be used together with other sources. This is especially true in cases where discovery of an agent is triggered by reports of symptoms or disease.

Interventions such as facility closure or administration of medical countermeasures may be warranted based on other types of information, even if preliminary environmental results are negative.

Most environmental sampling is iterative: a second and even a third sampling round are sometimes needed, and initial assessment sampling in response to a biological event is no different. Two follow-up sampling steps may be used to address uncertainty.

Step 1: Update incident details and perform additional targeted sampling

Investigators should update incident details by going back to law enforcement and public health sources to learn whether there have been any new developments. These new details can be incorporated into the supplemental sampling plan. In addition, discussions with supervisors and peer investigators may yield ideas and locations for a new targeted sampling.

Step 2: Augment targeted sampling using two additional sampling strategy options

"Full-inspection" sampling. Full-inspection sampling (Table 1) involves taking additional samples from each sampling location to minimize uncertainty. For example, consider the situation given earlier at the Megalopolis Airport which involved baggage. Targeted sampling might be used to focus on the one or two baggage carousels considered most likely to have been affected. However, if there are gaps in information about likelihood, a full-inspection approach would involve sampling the most likely contaminated location for every conveyor route and baggage carousel. Composite approaches can be used together with full-inspection strategies to maximize the proportion of the surfaces that can be sampled.

"Probabilistic" sampling. Probabilistic sampling involves dividing up the total area by using a grid. Once this is done, the grid spaces are numbered and then some of the grid spaces are randomly selected for sampling. The grid proportions and numbers and the number of samples

Table 1. Sampling strategies

Strategy	Description
Targeted sampling	Sampling locations and pathways considered "most likely to be contaminated" based on incident details and expert judgment
Full-inspection sampling	Sampling every location that falls under a particular pathway or area of concern based on incident details and expert judgment
Probabilistic sampling	Randomly sampling a portion of all locations based on a statistical design

depend on underlying assumptions and the desired level of confidence. Probabilistic sampling offers the benefit of allowing statistical inferences to be made about the likelihood of contamination. It typically requires larger numbers of samples and also requires additional time for "gridding out" areas and completing all analyses prior to drawing conclusions. This option is particularly valuable in cases where little or no incident information is available for targeted sampling and/or the contamination mechanism was such that every surface had an equal chance for contamination (i.e., no surface is more likely to be contaminated than any other). A number of systems are under development to assist with statistical sampling approaches, including the Building Restoration Operations Optimization Model (BROOM) (15) and the Visual Sample Plan (VSP) (14).

If results are positive, what happens next?

Surface sample results that are valid, positive, and obtained via initial assessment sampling with LRN analysis generally indicate that an event did occur, contamination is present, and persons in the area may have been exposed. At this point, the event moves to the next phase: characterization sampling. There may be exceptions to this generalization. For example, additional questions and review may be needed for some agents (e.g., the bacterium causing tularemia) and some locations (e.g., rural or some urban settings) where background contamination is plausible. These require additional review and consultation.

The emergency nature of initial assessment sampling may limit the ability to produce a comprehensive written report immediately after the sampling is completed. However, it is very important that investigators write their reports as soon as possible to document sampling strategies and fully describe sampling locations, numbering conventions, and sampling methods for those that follow.

Fictional airport scenario: initial assessment developments

The surface samples taken from the suspicious suitcase came back positive for *B. anthracis*. The FBI estimated that the device was expelling aerosol for several minutes before being discovered at Terminal 2 baggage carousel 4. The passenger list for flight 49 showed that the bag had been checked by a Mr. Adam Smith and that someone had checked in and occupied the seat in question. The entire Megalopolis International Airport was closed as a precaution until more investigations could be performed.

Your team arrived on scene at 6 p.m., knowing that there would be a long night ahead. News camera crews filming the evening news were already lining the road leading to the terminal. You were briefed on the incident details by law enforcement representatives, and you spoke with the airport's engineer and with the baggage handling crew. You questioned the engineer closely about the nature of the conveyor system, how and where

Terminal 2 connects with the other airport terminals, and how long the HVAC system ran before being shut down.

After suiting up and performing a walk-through, you identified at least three plausible contamination pathways:

1. The baggage unloading and conveyor process pathway
2. Foot traffic and cross-contaminated baggage associated with the passengers who picked up their luggage and departed before the suspicious bag was noticed
3. The airborne pathway, encompassing the baggage carousel area airspace and the airspace associated with the path of the baggage conveyor

Your sampling plan involves collecting surface samples from some other luggage found in close proximity to the suspicious tan suitcase, along with some from the conveyor belt and floor close to where the bag was found. You also collect some floor and conveyor samples at the location where the bags are initially transferred to the conveyor.

To evaluate the airborne pathway, you collect surface samples from the nearest ventilation intake ducts on the ceiling along with several samples from the TV screens used to show the flight numbers being serviced by each carousel. You also arrange to collect surface samples from the Terminal 2 HVAC system filters.

The samples yield several positive results, suggesting that the contamination did spread beyond the discovery area. Several bags had fairly high colony counts, and positive results were also obtained for samples from the floor and at the bag transfer conveyor location. A positive result was obtained on samples from the ceiling ventilation register and the carousel TV screen, confirming that aerosolization did occur. A positive result was also obtained for the TV screen for carousel 2, located about 60 ft away from carousel 4 and adjacent to the exits for the parking shuttles.

The public health response team members discuss your results and add the information to their other findings. They are trying to identify and define the group who should receive precautionary antibiotics, which in this case would be ciprofloxacin. They decide to include anyone who was in Terminal 2 during the specific period involved. A major concern is locating the 14 passengers who picked up potentially contaminated luggage and left the airport before the suspicious bag was noticed.

Characterization: What Is the Extent of Contamination?

Valid positive results and/or other key findings trigger the time-sensitive public health interventions associated with the initial response, such as provision of postexposure prophylaxis for those who may have been exposed and full or partial closure of the facility. The characterization phase, which involves seeking additional clues about the magnitude and spread of contamination to better understand the cleanup that will be needed, can then begin.

Fictional airport scenario: determining the spread of contamination

By the next morning, the situation at the airport is front-page news and the cable news channels are providing hourly updates. Many airport workers and members of the public were given antibiotics as a result of the initial investigation findings. Health departments are on high alert for any reports of anthrax-like symptoms, but so far no potential cases have been reported. Now the concern has shifted to cleanup operations and putting the unaffected parts of the airport back in operation. The facility operator wants to determine as soon as possible where the contamination is located and which areas can be reopened. The governor is also eager to see the airport safely reopened due to the significant financial impact of the closure on the local economy. How can you use environmental sampling to make these determinations? Given the modular nature of the airport with its four attached terminals, what is the likelihood that contamination has spread to other parts of the facility? How can the airport authority avoid the huge costs of keeping the airport closed unnecessarily while also preventing any additional contamination or exposure? While facility characterization is the primary focus, questions have been raised about other types of locations. What about the need to check on the luggage bay of the aircraft used for flight 49? The bay could be contaminated if the device began expelling aerosols while still on board. One of the airport rental car companies also called to request a vehicle be checked since it had been rented by one of the passengers who picked up baggage prior to the discovery of the device, and the company is concerned that the trunk might be contaminated. You add it to your ranked list of locations of concern.

Coordination and handoff issues from the initial assessment

A handoff may occur as this phase begins. The public health investigators involved with the initial assessment (e.g., from the CDC's National Institute for Occupational Safety and Health and/or state public health departments) are likely to be departing, and environmental on-scene coordinators (e.g., from the Environmental Protection Agency [EPA] and/or state environmental agencies), cleanup contractors, and consultants working for the facility owner are arriving to perform characterization and cleanup work. A face-to-face meeting can ensure continuity at the handoff by addressing methods used, contamination locations and pathways, uncertainty issues, and sample-naming conventions.

The characterization phase also marks the need to begin coordinating among the various regulatory agencies such as the EPA. The EPA provides guidance to facility owners and their consultants on the approaches needed for remediation and the preparation of a written remedial action plan (RAP) to spell out remediation plans in detail for discussion, review, and approval. The characterization phase sampling results from the remediation planning process are typically included in the RAP report. The RAP also spells out sampling plans for any remaining characterization and clearance sampling as well as criteria for judging the success of the remediation. While this chapter does not discuss remediation options in detail, large-scale remediation efforts after a terrorism event are complex and require careful preparation and coordination.

Environmental sampling objectives

The objective of characterization is to systematically expand on initial assessment findings to identify other contamination locations until the extent of contamination is understood and to determine the magnitude of contamination at the affected facility. Contamination spread can be affected by a number of factors ranging from the size and characteristics of the particles, the conditions associated with the initial release, and the existence of conditions that can lead to resuspension of settled particles.

Characterization is used to understand the contamination footprint at the facility, especially the boundaries of contamination. How many rooms are contaminated? How extensively has the ventilation system been contaminated? If machinery is present, was it contaminated? Characterization takes into account the specific agent involved in addition to the scenario and site-specific details. As mentioned, the resulting information will shape planning and implementation of the cleanup.

There is some overlap between initial assessment sampling and characterization sampling. In most cases, the public health sampling performed during the initial response marks the beginning of the effort to determine the scope of contamination. This sampling is done to identify the subpopulations at a facility that might need to receive medical countermeasures. It is not performed to identify all discrete surface locations where contamination is present. While its results are meaningful, thorough characterization sampling needs to address the spread of contamination in more detail because the results are used to direct the cleanup activities.

How do remediation options affect characterization?

There are two basic remediation strategies: targeted remediation and full remediation. Targeted remediation involves cleaning only the contaminated locations and their immediate environments, whereas full remediation involves cleaning all surfaces. Fumigation is an example of a full remediation. The choice of remediation strategy should affect the level of effort expended on characterization as described in this section.

Targeted remediation requires a high level of confidence that contaminated locations are known. It requires a full-characterization effort, where every positive result is followed up by additional sampling in the perimeter area surrounding the location where the positive sample was found. Any newly found positive sample then triggers an additional round of wider perimeter sampling until no further positive samples are found. The picture provided by these results is integrated back into the understanding of the contamination pathways, and follow-up sampling may be needed to explore any findings that suggest unexplained or inconsistent pathways. Full characterization is important because if only known contaminated areas are cleaned, any undiscovered contamination presents a threat to the health of returning occupants. The Hart Senate Office Building is an example of a case where a targeted remediation was used.

Full remediation is performed for virtually all surfaces; therefore, it reduces the need for a full characterization. Partial characterization needs to be performed to identify and understand the contamination pathways and heavy contamination patterns, but not every positive result triggers additional sampling in the wider perimeter area as with full characterization. The fumigations performed at two of the U.S. Postal Service facilities that sorted the 2001 letters are examples of cases where full remediations were performed.

In sum, there is a trade-off between characterization and remediation. The narrower the scope of the remediation, the wider the scope and effort needed for characterization sampling and vice versa.

Where to sample? Developing a characterization sampling strategy

As seen from the above discussion, characterization sampling builds on the initial assessment findings. It uses an iterative targeted sampling strategy to follow up on perimeter surfaces near the locations of previous positive samples to find the contamination boundaries, taking care to follow all plausible pathways. For example, if initial assessment sampling identified foot traffic as a pathway and positive samples were found on floor carpets, characterization could further follow up to check floor areas of offices associated with individuals identified as having visited the area where the bioterrorism incident occurred. As with initial assessment, there may be a need to supplement targeted strategies with probabilistic or full inspection sampling approaches where needed. Probabilistic sampling may be helpful to increase confidence in cases where targeted remediation strategies are being proposed. A "full" inspection sampling strategy offers another alternative option. For example, foot traffic was a known contamination pathway at the Hart Senate Office Building, and the floor area inside each and every doorway was sampled as part of a full inspection characterization sampling effort.

Special issues

Use of PCR. PCR analysis is sometimes used during characterization to facilitate the analysis of large numbers of samples and to provide near-real-time results. It is appropriate to use PCR to supplement culture-based results for characterization since viability and confirmation of results are not as critical as during initial assessment or clearance. See chapter 9 for further details about PCR.

Semiquantitative results. Standard estimation of the number of CFU provides an additional layer of information to understand contamination patterns. Differentiating between lower and higher contamination levels allows important insights into contamination pathways. While it is important to understand that there are no hard and fast guidelines for judging risks posed by surface contamination levels, these semiquantitative results do provide important clues to how contamination occurred. The results may also be important for planning remediation strategies.

Item disposal. It is common for remediation staging and setup to begin during the same period as characterization. Decisions may be made to remove and dispose of certain items instead of remediating them. Some surfaces may be stripped and smaller items may be sent off-site for remediation at a commercial ethylene oxide sterilizer or other appropriate facility. It is important that the locations of removed contaminated items be noted so that perimeter characterization sampling can be completed. In addition, it is important to view these preremediation removal activities as representing potential pathways of contamination. These pathways need to be sampled to ensure that the remediation process itself does not create additional unanticipated cross-contaminated locations.

Fictional airport scenario: characterization sampling developments

Characterization sampling yielded a number of positive surface samples in the first-floor baggage claim area, on the floor carpets of the nearby elevators, and on a number of HVAC return air registers. Contamination was also found on the second floor of Terminal 2, apparently spread by air currents passing between floors through openings such as the escalator passageways. Due to the evidence of contamination spread, the Megalopolis Airport Authority decided to proceed with a fumigation of Terminal 2.

Given the costs and delays associated with fumigating additional terminals, a decision was made to develop a probabilistic sampling strategy to comprehensively examine pathways in the vicinity of the Terminal 2 connections with Terminals 1 and 3. The goal was to provide more information on whether contamination spread via these connections into the adjacent terminals. Characterization was also performed for rooms traversed by the baggage conveyors.

A special team was created to focus on sampling of items and off-site concerns. Several positive samples were collected from the trunks of private and rental vehicles, and the four-bedroom home of one of the early-departure passengers was found to be contaminated, requiring additional remediation.

Clearance: Was Remediation Successful? Is the Space "Clean"?

Characterization sets the stage for cleanup. Remediation is then performed once conditions are ready and plans have been reviewed and approved. Clearance sampling is performed after remediation but before critical barriers are taken down. Critical barriers refer to asbestos abatement-type barriers made of suitable plastic combined with negative-pressure air filtration units to keep the affected area under negative pressure. Barriers may be used when a large area is broken down into several smaller areas for sequential remediation. Clearance sampling provides an objective determination that remediation was successful and the facility is ready for final preparations for reoccupancy.

Fictional airport scenario: remediation

A combination of remediation approaches were used to clean up the Megalopolis International Airport. Terminal 2 was sealed up and closed for 3 months for remediation. Fumigation was performed for the main terminal space and ventilation system, and targeted cleaning was done for several smaller areas, five automobiles, and several motorized baggage carts. The other three terminals were able to stay open, allowing most flights to continue.

Your team developed a multifaceted clearance sampling plan to address this combination of approaches. Some modifications were incorporated after review by outside reviewers working with the EPA and the local health department. Remediation went smoothly, and your team began collecting surface samples at the designated terminal locations shortly after the spore strip indicators showed that the fumigation design parameters had been met.

Coordination issues

Since clearance sampling is typically performed by the same group that handles characterization, coordination and handoff to a new sampling team is generally not necessary. However, as the overall project approaches completion, coordination with peers and subject matter experts may be needed. These individuals are assembled to serve on environmental clearance committees (ECC) to provide a final peer review of the effort. In addition, concerns from facility occupants and building owners may introduce issues that need to be addressed in clearance sampling. The National Research Council discussed the value of such input and included several recommendations about the need to include stakeholders in risk management decision-making for reopening public facilities (13).

Environmental sampling objectives

Clearance sampling is carried out to test the remediation effort to evaluate whether it was effective; it involves looking for evidence of residual contamination. Because remediation has been performed, the expectation is that all results will be negative. This is where the limitations and data gaps for current sampling methods are most obvious. While current methods are undergoing validation testing, this work is not complete; key information, such as the lower limit of detection, is not available. Thus, when results come back "negative," it actually means that no biological organisms were detected above a certain detection level, and while that level may be low, it is unclear how few organisms it actually represents. In light of these constraints and because investigations cannot be put on hold until methods are fully validated, clearance sampling strategies have been designed using the following framework:

- Use the best science and data available (for example, incorporate any new scientific developments that have improved available methods)
- Use the most sensitive sampling methods available
- Use a combination of sampling methods
- Use thorough and rigorous sampling strategies
- Use "no growth" as clearance criteria for judging success (no growth is equivalent to "zero" detectable CFU in a culture)
- Be explicit about limitations and uncertainty in communication and reports

How clearance sampling is different

In contrast to initial assessment and characterization sampling, clearance sampling involves a defined sequence of tests as described in this section.

For localized contamination and targeted remediations, the sequence includes a surface sampling step followed by an aggressive air sampling step. Aggressive air sampling originated as a testing method for asbestos abatement jobs. It involves using a leaf blower and fans to dislodge contaminants from any surfaces and force them into the air, where they are available for collection by an air sampling device. In biological sampling, this step is performed only after the surfaces have first passed the surface sampling test. Aggressive air sampling provides an additional level of testing for available biological agents and complements the surface sampling to provide an overall more rigorous test. Note that the testing is done before removing critical barriers and negative-pressure machines.

In cases involving extensive contamination and full remediation, such as where fumigation is more likely to be performed, the sampling does not commence until preliminary tests ascertain that fumigation conditions were met. For example, biological indicators are used to determine if suitable fumigation conditions were created. Surface sampling follows once it has been determined that fumigation conditions met the testing criteria established in the RAP. Aggressive air sampling then follows once negative surface sampling results are received. All samples are subject to culture analysis using LRN methods for reliability.

Where to sample? Developing a clearance sampling strategy

Clearance sampling strategies address the following questions:

- Are previously contaminated locations now "clean"?
- What sampling is needed in other areas to assure that those surfaces are now "clean"?

Clearance sampling strategies integrate both judgmental and probabilistic approaches into an overall strategy as follows.

1. *Focused sampling*. The goal of focused sampling is to resample every discrete location where a positive contamination result was found in order to verify that it is clean. The rationale is that previously contaminated locations represent the most challenging test of remediation effectiveness. While these locations would have been targeted for cleaning and sampling, it is still important to resample them to provide an objective determination that they are clean and test negative. Remediation techniques are not perfect, and there have been cases where a location was found to test positive even after remediation.

2. *Biased sampling*. The goal of biased sampling is to target locations more likely to have been contaminated, such as surfaces along contamination pathways. This type of sampling is also important in cases where a limited characterization was performed because the actual contamination boundary may not have been determined. Biased sampling can also be used to target surfaces that are deemed more important than others, such as those with a higher likelihood of employee contact on reoccupation.

3. *Grid/random sampling*. The goal of grid sampling is to systematically sample other spaces. The rationale is the need to address other areas of the facility that could have become inadvertently contaminated during remediation activities. Grid sampling involves collecting a specified minimum number of samples in every grid location or space depending on its size. Locations within each grid space may be chosen at random. Grid sampling is important in cases when full remediation of an entire building requires clearance sampling.

True probabilistic sampling allows statistical inferences to be made and allows a defined degree of statistical confidence in the negative results (8). While there are a number of practical challenges to probabilistic sampling, it is nonetheless important to incorporate this approach. Statistical sampling requires inputs for detection limits, risk criteria, and size of hot spots, and these values are currently under development. Sampling plans using probabilistic sampling may require large sample numbers depending on the sample designs and the level of confidence desired.

What if clearance testing finds a positive sample?

Any positive findings during clearance sampling will trigger some amount of further cleaning and re-evaluation prior to a new round of testing. The measures to be taken (from biological indicators to surface sampling to aggressive air sampling) in the event of a positive result are described in the team's sampling and analysis plan.

Reporting results

The team performing the clearance sampling reports results and may present its findings and methods to the ECC group reviewing the entire remediation effort. The ECC group in turn typically provides a written clearance statement to the facility owner and/or the local health authorities with jurisdiction over facility occupancy.

Fictional airport scenario: the end

The Megalopolis Airport Authority and the local health department jointly announced the reopening of Terminal 2 6 months after the incident took place. The decision was based on the technical determination received from the 15-member ECC that the remediation effort was successful. Your team provided an extensive briefing for the ECC to describe the many important aspects of the clearance sampling plan: sampling strategy components ranging from resampling original contaminated locations to probabilistic sampling; the variety of surface and air sampling methods used and their strengths and limitations; and the rationale for the sequential order of the sampling. Several positive clearance samples were obtained in one restricted baggage conveyor, requiring a round of recleaning and retesting per the provisions of the RAP. The other samples, over 2,000 in all, all tested below the detection limit. The overall plan worked well and provided the information and level of confidence the ECC needed to do their job.

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Sampling for Biological Agents in the Environment

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