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PAPER

CRIMINALISTICS

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Guidelines for the Identification of Unknown Samples for Laboratories Performing Forensic Analyses for Chemical Terrorism*

ABSTRACT: Since the early 1990s, the FBI Laboratory has sponsored Scientific Working Groups to improve discipline practices and build consensus among the forensic community. The Scientific Working Group on the Forensic Analysis of Chemical, Biological, Radiological and Nuclear Terrorism developed guidance, contained in this document, on issues forensic laboratories encounter when accepting and analyzing unknown samples associated with chemical terrorism, including laboratory capabilities and analytical testing plans. In the context of forensic analysis of chemical terrorism, this guidance defines an unknown sample and addresses what constitutes definitive and tentative identification. Laboratory safety, reporting issues, and postreporting considerations are also discussed. Utilization of these guidelines, as part of planning for forensic analysis related to a chemical terrorism incident, may help avoid unfortunate consequences not only to the public but also to the laboratory personnel.

KEYWORDS: forensic science, chemical terrorism, unknown, unknown samples, identification, sample acceptance, analysis, reporting

Chemicals associated with terrorism can present acute hazards not normally encountered during routine operations by laboratory personnel. Appropriate caution and safety should be exercised when dealing with these types of hazardous materials. The analysis of unknowns is typically a complex process even for samples not contaminated with chemicals used for terrorism (1–15). Consequently, failure to plan for forensic analysis before an emergency might lead to unfortunate consequences not only to the public but also to the laboratory personnel. As part of planning for such an incident, laboratories should prepare guidance document(s) specific to their needs and capabilities, including the development of suitable criteria for the acceptance of samples and development of an analytical testing plan. The guidance document(s) should be regularly reviewed and updated to meet the changing needs and capabilities of the laboratory.

To help with this planning process, the Scientific Working Group on the Forensic Analysis of Chemical, Biological, Radiological and Nuclear Terrorism (SWGCBRN) approved guidelines that were developed by the Scientific Working Group on Forensic Analysis of Chemical Terrorism (SWGFACT). Since the early 1990s, the FBI Laboratory has sponsored Scientific Working Groups to improve discipline practices and build consensus among forensic community partners (16). These guidelines provide guidance on issues forensic laboratories encounter when accepting and analyzing unknown samples associated with chemical terrorism. It also describes some of the practices that a laboratory should follow when investigating unknown chemical terrorism samples.

This manuscript presents these guidelines to provide the forensic laboratory with information on how to address some of the common questions that arise when analyzing unknowns resulting from suspected or actual chemical terrorism incidents. Each

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situation and analysis will be different, so the laboratory should prepare to meet the corresponding needs and challenges. The information provided in these guidelines is largely intended to be a planning tool for laboratories that may need to provide a forensic analytical response in the case of an unknown threat or incident, not a "how-to" manual for use during the actual incident. It is meant to complement the experience and professional judgment of the laboratory personnel as well as to provide potential solutions to the challenge of the identification of unknown samples.

As this document is guidance, laboratories can give consideration to alternate approaches of achieving the intent of these guidelines. Regardless, it is assumed that the laboratory is operating under a quality assurance system, such as the one described in the *Quality Assurance Guidelines for Laboratories Performing Forensic Analysis of Chemical Terrorism* (17) and has a program for the validation of analytical approaches, such as the one described in *Validation Guidelines for Laboratories Performing Forensic Analysis of Chemical Terrorism* (18). Specifically, this document addresses the following types of questions that surround the analysis of an unknown sample by a forensic chemistry laboratory:

- What is an unknown sample?
- What information or intelligence is available regarding the unknown sample?
- Is the laboratory capable of safely handling the unknown sample?
- What are the analysis objectives?
- Does the laboratory have the capabilities of fulfilling the analysis objectives?
- What is the laboratory's analytical testing plan for the unknown sample?
- What constitutes definitive and tentative identification?
- How, when, and to whom should the laboratory report the results?

Defining Unknown Samples

As with most discussions of complex processes, it is important to carefully define key terms. Table 1 contains some key definitions for terms used in these guidelines. While other terms carry their standard definitions from forensic science and metrology, some may be more appropriately defined in two SWGFACT guidance documents (17,18). The most complex definition is that of the "unknown sample" itself, and it is important to briefly discuss what an "unknown sample" is, and what it is not.

TABLE 1—*Some definitions of terms in these guidelines.*

Laboratory	A facility in which analysis associated with chemical terrorism is performed
Analytical procedure	An orderly step-by-step instruction designed to ensure operational uniformity and to minimize uncertainty
Client	An individual or organization requesting an analysis
Field safety screening	Activity conducted outside the laboratory to assess potential hazards
Field testing	Analytical activity conducted on the sample prior to its arrival at the laboratory, including field safety screening
Reference material	Material for which component identities, types, or values are certified by technically valid procedures and is accompanied by or traceable to a certificate or other documentation that is issued by a certifying body
Standard	A substance of known identity and purity and/or concentration

For the purposes of this document, an unknown sample is one that is associated with a planned, threatened, or actual act of chemical terrorism and whose identity has not been established. Samples may range from single-component materials to a mixture of components in a complex matrix. Examples include suspicious powder within an envelope, liquid seized in a clandestine laboratory, soil from an area where mass illness has been reported, a food additive that is suspected of being tainted, air collected near the site of an explosion, water that is rumored to be poisoned, and clothing from victims with symptoms of chemical exposure.

In the context of this document, unknown samples are collected for forensic analysis, not for other reasons such as regulatory monitoring and compliance. For example, samples originating from remediation (clean up) activities are not considered unknowns because they involve targeted analysis. Also, this document does not address the analysis of chemical warfare agents, biological agents, radiologicals, or explosive unknowns, which are legally analyzed only by specialized laboratories with appropriate authority and/or specialized equipment. However, the potential presence of these materials needs to be taken into consideration prior to acceptance of the sample into the laboratory. Biological agents are listed in the Select Agent Registry (19). Chemical warfare agents are listed in Schedule 1 according to Chemical Weapons Convention (20).

Considerations in the Acceptance and Analysis of Unknowns

Gathering Information and Intelligence About Unknown Samples

Before the laboratory accepts an unknown sample for analysis, it is important that the laboratory gathers as much information as possible about the sample from the client, investigator, or any other reliable source of intelligence. Each laboratory should develop its own sample acceptance policy for unknowns, based on the questions such as those summarized in Table 2, which reflect issues that should be addressed before an unknown sample is received by the laboratory. The information obtained may determine whether the sample may be accepted, and this information is in addition to that which usually accompanies traditional forensic samples.

Laboratories should decline to accept samples that do not meet their policy for sample acceptance (e.g., chemical warfare agents, biological agents, radiological materials, or explosive unknowns). Sample acceptance may depend on field testing results; however, the laboratory should be aware of the limitations of any previous tests performed on the sample. This information may be useful for the design of the analytical testing plan. Regardless of what field testing has been conducted, the laboratory should perform any safety screening tests it believes necessary to ensure safe handling and analysis of the sample.

In addition to affecting sample acceptance, information and intelligence about the sample may influence the course of the analysis. Information and intelligence of chemical terrorism might include evidence of a clandestine laboratory, records of chemical reactions or formulae, or reports of a suspect with a history of chemical knowledge. This information should not unduly bias or limit the analysis plan.

The laboratory should also keep in mind that some of the information that the investigators or samplers possess may be classified and therefore cannot be shared with the laboratory unless laboratory personnel have the appropriate security clearances. Therefore, the laboratory may want key personnel to obtain security clearances appropriate to potential client expectations.

TABLE 2—*Topical questions prior to unknown sample receipt by laboratory.*

Information available at the scene and by the investigation	Are there any obvious indications of adverse effects on health or the environment?
	What are the results of field screening for chemical warfare agents, biological agents, radioactivity, and explosives?
	Have explosive devices been rendered safe?
	Is there any evidence at the scene or gathered by investigation that would help identify the unknown?
	Is there any information about or from suspects that might assist in the analysis?
Information about the sample	What are the field testing results that might help identify the unknown, and what are the limitations regarding these results (e.g., source and quality)?
	Have other laboratories analyzed the sample, and what were the results?
	Are there multiple hazards present?
	Can the laboratory separate the sample's components without destroying forensic evidence?
	What is the size and type of the sample and can it be received by the laboratory?
	Is the sample packaging appropriate?
	Are field control samples available?
	What documentation is available with the sample that would assist with the analysis?
Information related to client expectations	Does the laboratory's analytical testing plan conform to the expectations of the client?
	Does the laboratory have the technical capability and laboratory capacity to meet the submitted request?

Ensuring Safety in the Laboratory

The laboratory should avoid extending the consequences of the terrorist incident to the laboratory staff and facility. To ensure the safety of the laboratory staff, it is essential to have and follow a documented environmental health and safety program. This program may include medical surveillance and should be in accordance with federal and state laws. As stated in the SWGFACT *Quality Assurance Guidelines for Laboratories Performing Forensic Analysis of Chemical Terrorism* (17), this program should include a documented ongoing safety-training component for laboratory personnel. The laboratory should identify a person as the safety officer and ensure they have appropriate training.

After the sample has been accepted, subsequent analytical results and/or newly obtained intelligence information may suggest the presence of a component that the laboratory is not equipped to handle. The laboratory should then discontinue the analysis, secure the sample, and immediately contact the client and/or an appropriate authority.

Establishing Analysis Objectives

The analysis objective(s) should be agreed upon with the client before beginning the analysis. Analysis objective(s) may change during the laboratory investigation. The primary objective will generally be to identify hazardous components associated with the sample. Related objectives may be to quantify the amount of the identified chemical or establish the absence of targeted hazardous substances at relevant levels, such as in the case of a potential hoax. If no hazards are identified within the sample, it may still need to be characterized further for investigative purposes, such as source attribution.

The size and number of samples may limit the objectives or the sequence in which the analyses can be performed. If only a limited amount of sample is available, it may be necessary to choose between identifying several chemical contaminants and saving some of the sample for additional analyses.

Depending on the situation, other investigatory priorities may complicate analytical objectives. For example, the client may require that an amount of sample be returned or retained. The sample may also require special handling during analysis if other examinations need to be performed, for example, fingerprint or DNA testing. The laboratory personnel should discuss potential complications with clients prior to handling the sample.

Capability of Laboratory to Fulfill Analysis Objectives

The laboratory should review its technical knowledge, experience, and instrumentation and develop a general analytical strategy to serve as a basis for the analysis of unknowns. The guiding principle behind developing the analytical strategy is that information obtained from previous steps in the process should direct subsequent analyses. Figures 1–3 contain example strategies for liquids, solids, and gasses, respectively, which the laboratory might use to develop its general strategy. The laboratory's general strategy may differ in complexity than the examples in Figs 1–3.

Figures 1–3 refer to analytical techniques frequently utilized for forensic analyses. Other techniques are available and may be very useful for a particular sample. Factors that need to be considered during the selection of an analytical technique include limit of detection, precision, accuracy, and selectivity, as discussed in SWGFACT *Validation Guidelines for Laboratories Performing Forensic Analysis of Chemical Terrorism* (18). In selecting any analytical technique to fulfill analysis objectives, laboratories should actively seek to understand the technique and consider its limitations and associated sample preparation requirements. Much detailed information is available in standard references such as textbooks. However, as a general sample preparation note specific to the analysis of unknown samples, it is worthwhile to consider that when analyzing an unknown sample that is potentially neat or highly concentrated, the sample should be diluted 100- to 1000-fold before many analyses. If results are negative, the sample can be analyzed with less dilution, or with no dilution.

A laboratory may find it useful to compile a table summarizing the analytical techniques utilized in their analytical strategy, along with the techniques' limitations and associated sample preparation requirements. This type of table can serve several important purposes. For example, it can assist the laboratory with the identification of its own capabilities and limitations. It can also provide a means of communicating these capabilities to its clients. Table 3 contains an example format for two analytical techniques. An expanded version of Table 3, containing information for all analytical techniques in Figs 1-3, can be accessed via directions which appear at the bottom of Table 3.

The laboratory should also evaluate its capabilities in the context of the pressure (e.g., political and/or social), which may be unique to a terrorism incident, including rapid turn-around time and surge capacity. Based on changing circumstances during the incident, the laboratory may have to reassess its capability to meet analysis objectives. In meeting these pressures, the laboratory should continue to follow the SWGFACT *Quality Assurance Guidelines for Laboratories Performing Forensic Analysis of Chemical Terrorism*, including chain-of-custody and evidence control (17). If the laboratory does not have the capability to fulfill the new analysis objectives, the client should be contacted and/or the analysis objectives should be reexamined.

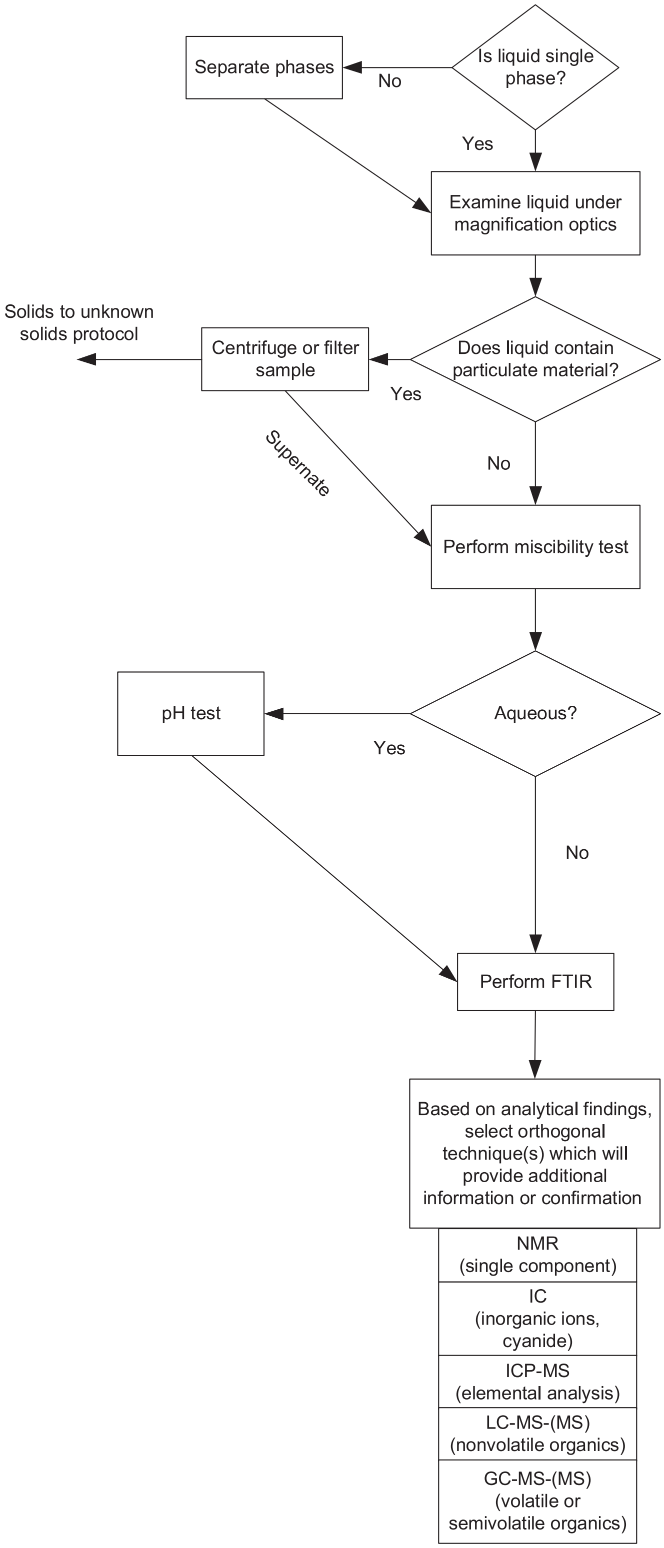

FIG. 1—*Flow chart for the analysis of an unknown liquid sample.*

Analytical Testing Plan

Prior to initiating any analysis, the laboratory should develop an analytical testing plan. Normally, the laboratory will adapt its general analytical strategy to meet the analysis objectives. This may involve planning the sequence of analyses to give priority to certain objectives for reasons such as the sample characteristics and amount of sample available. In the course of this planning, the feasibility of meeting the analysis objectives may need to be discussed with the client, and the testing plan revised accordingly. In the course of implementing the testing plan, it may become apparent that the plan needs to be revised in response to the results of ongoing analysis.

The plan should include the following elements: (i) analytical objectives, (ii) adaptation of a general analytical strategy to meet the analytical objectives, (iii) quality assurance and method validation measures to be employed, and (iv) possible limitations of the analytical techniques selected for the plan.

Identification

Identification can be either definitive or tentative. Well-defined criteria for what constitutes definitive or tentative should be established. The scientific literature abounds with examples defining identification criteria for particular purposes, leading to frequent discussions of the topic (1,4,6,7,10,11,15,21). Different organizations, such as Organization for Prohibition of Chemical Weapons (22), Environmental Protection Agency (12), Society of Forensic Toxicologists (9), European Union Commission (5), World Anti-Doping Agency (14), and Scientific Working Group for the Analysis of Seized Drugs (8), have defined different requirements for identification criteria specific to their purposes. However, the following guidelines regarding definitive and tentative identification are applicable for the identification of unknowns associated with chemical terrorism.

Definitive identification is based on a combination of techniques that both demonstrates the presence of the specific chemical and minimizes false-positive identification. At least two consistent results from orthogonal techniques involving reference materials or standards should be obtained. At least one of these techniques should be structure elucidating when possible given the nature of the analyte (e.g., for certain inorganic species it will not be possible). "Hyphenated" techniques are considered in these guidelines to yield one result. For example, GC-MS with unit mass resolution with a single ionization mode or chromatographic column phase is considered one result. Therefore, a second result should be obtained using an orthogonal technique.

Tentative findings indicate the likelihood that a particular chemical is present. Definitive identification may not be possible, yet a tentative identification may benefit the client (e.g., by providing an investigative lead). While tentative identification also requires at least two consistent results from orthogonal techniques (if possible given the nature of the analyte), time constraints in an emergency or lack of available reference materials, standards, or appropriate techniques may preclude definitive identification. Validated databases and libraries may be helpful when a standard is not available. However, when using databases and libraries, review of the spectral matches by competent personnel is necessary for a tentative identification. If a tentative identification is made, appropriate efforts should be made to subsequently perform a definitive identification, for example, when time constraints are resolved or a standard becomes available.

Reporting Results

The laboratory and client should agree on single points of contact to discuss results to avoid confusion or improper release of information. In a terrorism situation, there may be intense pressure to deliver results. Inaccurate, incomplete, and/or improperly reported analytical results might either falsely reassure the public or cause

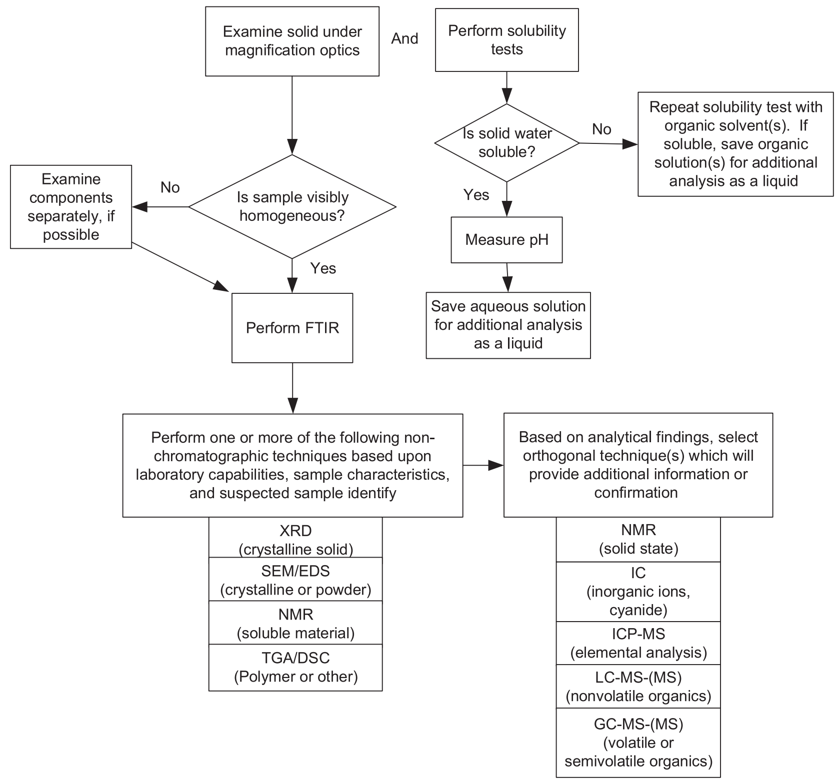

FIG. 2—*Flow chart for the analysis of an unknown solid sample.*

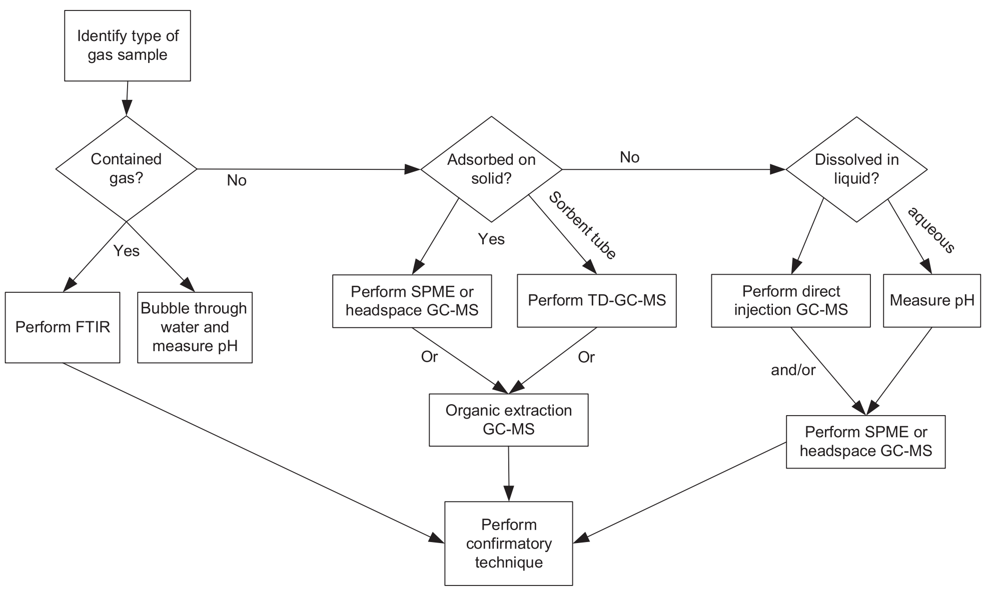

FIG. 3—*Flow chart for the analysis of an unknown gas sample.*

unnecessary panic. When reporting results, the following questions should be considered: (i) To whom will the report be directed? (ii) Have appropriate security classification issues been considered? (iii) Has the client's request been addressed, including both positive and negative results? (iv) Have the established analysis objectives been met? (v) Have all reporting requirements been met, for example, as outlined in the SWGFACT *Quality Assurance Guidelines for Laboratories Performing Forensic Analysis of Chemical Terrorism* (17)? (vi) How confident are the identifications? (vii) What are the uncertainties associated with quantifications? (viii) Are there limitations in the analysis? (ix) Can interpretative statement(s) provide clarity to the results? and (x) Are expert remarks or conclusions appropriately included in the report?

Considerations Following Reporting of Analysis of Unknowns

After the laboratory has completed its analysis, it is important to consider that the laboratory's involvement may not end upon

TABLE 3—*Example entries for table of laboratory-specific analytical techniques.*

Activity/Analysis	Applicable Physical State	Information Obtained	Limitations	Sample Preparation
Fourier transform infrared spectroscopy (FTIR)	Solid Liquid Gas	FTIR provides structural information about compounds, by identifying functional groups that are present Bulk material can be easily classified using FTIR. The bulk composition of a gas may be rapidly determined by FTIR equipped with a gas cell. This technology may also detect the functional groups at part-per-million levels or higher of other components This is a nondestructive technique	Trace components of a mixture may not be detectable by FTIR Some inorganic materials may not absorb IR radiation Library searching may be helpful in identifying unknowns, but the search is dependent upon the library's quality and analyst's experience Detection of multiple components may require separation of the sample's components by a chromatographic method or other means	None needed if using an ATR attachment for bulk analysis If solid samples can be mechanically separated, small particles can be analyzed individually using an FTIR microscope attachment Liquids may be evaporated, and any remaining residue may be analyzed by FTIR. Liquid extraction and SPE may also prove beneficial with mixtures When dealing with chemicals that are potentially toxic, the FTIR should be used in a chemical fume hood
Physical examination	Solid Liquid Mixed phase	Determine physical state of sample. Identify obvious mixtures	Not all solids that appear to be homogeneous are pure materials	None needed

ATR, attenuated total reflectance; SPE, solid phase extraction.
The complete version of this table can be obtained via the contact for additional information listed below or at http://www.epa.gov/nhsrc. Search for "Identification of Unknown Samples."

submission of the report. Future laboratory involvement may include: (i) return of sample to the client or transfer of a sample aliquot to another lab, (ii) requirement for testimony by laboratory personnel, (iii) requests for additional analyses, (iv) requirement for long-term storage relative to sample stability, regulatory limitations, and facility storage capacity, (v) requirements for additional security or safety controls for hazards revealed by analyses, (vi) disposal or destruction of wastes in compliance with regulations, (vii) interpretation of inconclusive results, and (viii) suggestions for future analyses.

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