

(U//FOUO) Assessing Biological Laboratories at Unconventional Sites

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(U) Scope

(U//FOUO) Law enforcement officials and first responders may encounter biological laboratories or scientific equipment and materials at unconventional sites such as private residences or businesses not normally associated with biological activities. The accompanying report, *Distinguishing Characteristics among Types of Biological Laboratories*, was prepared for DHS by the Lawrence Livermore National Laboratory and provides information to help assess the type of activity occurring at these sites.

(U) This product was prepared in support of DHS and FBI activities and to assist federal, state, local, and tribal government agencies and authorities, the private sector, and other entities in developing priorities for protective and support measures relating to an existing or emerging threat to homeland security.

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(U) Encountering Biological Laboratories at Unconventional Sites

(U//FOUO) Many innocuous reasons exist for the possession of some types of biological agents and associated laboratory equipment. For example, hobby, educational, or artistic uses such as home brewing or pilot-scale biotechnology research may include the same or similar equipment used in the malicious production of pathogens (see Figure). In some instances, however, the presence of a biological laboratory at an unconventional site could be an indicator of possible intent or capability to conduct bioterrorism.



(U) Figure. Home brewing kit.

— (U//FOUO) Recent media reports indicate that home experimentation with biological materials is becoming an increasingly popular activity. As a result, law enforcement officials and first responders may increasingly encounter biological laboratories in unconventional settings, such as residences. Examples of this trend—often referred to as amateur molecular biology, do-it-yourself biology, or "biohacking"—include biofuel research, genetic testing, and the genetic modification or manipulation of microbes.

(U//FOUO) Testing by trained professionals may be necessary to identify the type of organism being produced at an unconventional site and to determine whether it is hazardous or innocuous. Barring obvious red flags, such as instructions for the production of a harmful agent, indicators that could distinguish an illicit or harmful lab from a benign one are limited.

 (U//FOUO) The possession of scientific equipment and reagents such as enzymes, culture media, and many other chemicals or biochemicals used in biology, even at unconventional sites, is not regulated by Select Agent laws (see text box).

(U) Select Agent Registry Program

(U) The National Select Agent Registry Program, operated by the Centers for Disease Control and Prevention, oversees the possession of biological agents and toxins that have the potential to pose a severe threat to public, animal, or plant health, or to animal or plant products. The unregistered possession of a Department of Health and Human Services- or Department of Agriculture-designated select agent or toxin is illegal, with three exceptions:

- (U) Possession of a select toxin in its natural unrefined or unprocessed state—for example, possession of castor beans—is legal. Once a process intended to produce the toxin begins—for example, removing the ricin from the castor beans—it is regulated under the select toxin laws.
- (U) The possession of certain amounts of select toxins, on the order of milligrams, is legally allowed, with the exact amount varying according to the toxin. If a legitimate reason for the possession of a select toxin exists, these restrictions do not apply.
 - (U) Some less virulent strains of select agents do not require regulation under the Select Agent Program but may fall under the National Institutes of Health's *Guidelines for Research Involving Recombinant DNA Molecules*.

(U//FOUO) Biological materials and equipment or facilities, ranging in sophistication from educational or hobby kits to homemade equipment to advanced biological research labs, might be encountered in the field. Although advanced labs such as those with equipment designed specifically for working with bacteria and viruses are better suited for the production of harmful biological agents, simple equipment also can serve this purpose.

- (U//FOUO) Educational or hobby kits often include a microscope and basic bacterial or plant production supplies such as agar plates, growth media, and glassware. These could be part of an illicit lab, but are insufficient by themselves to be used for most purposes that would pose homeland security risks.
- (U//FOUO) Home brewing and winemaking equipment, such as large containers for fermentation, can be converted to produce harmful bacteria. This activity, however, may have characteristics that differ from beer and wine production. For example, if *Clostridium botulium*, the causative agent for botulinum, was grown using such equipment, the setup would produce a rotting odor instead of a yeasty beer smell or fruity wine smell and also would require bacterial agents and materials not used in beer or wine production.
- (U//FOUO) The high cost of traditional laboratory equipment has sometimes led amateur biologists to build unconventional laboratory equipment out of household items. Examples include: 1) a kitchen blender and salad bowl combination utilized as a centrifuge; 2) an electrophoresis box constructed from epoxy-sealed plastic toy blocks; 3) large light bulbs utilized as culture flasks. While homemade laboratory equipment items can usually be readily identified because of their placement in non-standard locations or use in a non-traditional manner, determining their function can be difficult and may require expert assistance.

— (U//FOUO) More advanced biological labs would enable a user to grow bacteria or viruses, produce and refine toxins, or genetically modify pathogens. These types of labs likely would include specialized equipment for specific tasks, such as an incubator for viral cell culture, a commercial fermentor for bacteria culture, separation columns for protein purification, or electrophoresis equipment for nucleic acid or protein separation.

(U) The attached report has a detailed description of the different types of biological labs, typical equipment, and potential legitimate and illicit uses.

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Distinguishing Characteristics Among Types of Biological Laboratories

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Executive Summary

This guide provides an overview of different biological laboratory types, with a focus on equipment and material commonly used in the labs. It primarily is meant to help law enforcement personnel or other first responders rapidly assess the type of activity occurring at a suspect biological laboratory, particularly one located in an unusual location, such as a residence. A key challenge is differentiating between a suspect lab used for harmless or legitimate purposes and one used for harmful or illegitimate purposes despite significant material and equipment commonalities. Barring obvious red flags (e.g., the presence of instructions for growing a harmful biological agent), distinguishing conclusively between a "good" lab and a "bad" one likely will involve testing and other investigative techniques. Nonetheless, initial informed observation of the equipment and material at a suspect site can help identify the general type of activity occurring, and combined with interviews of persons on-site, may reveal consistencies or inconsistencies between the stated purpose and capabilities of equipment and material observed. While this guide is intended to inform, it is not a replacement for direct expert assistance.

Overview of Biological Laboratory Types

Table 1 lists several biological laboratory types that might be encountered in the field and provides information about the equipment, material, utility, and potential applications for each lab type. We also have included a basic chemistry lab for comparison. Biological activities require certain lab types or lab capabilities and, by extension, certain types of equipment and material. For example, the growth of anthrax most likely would require a basic microbiology lab set-up, and thus equipment like flasks, growth media (i.e., liquids used to support the growth of cells or microorganisms), and agar plates (essentially a sterile Petri dish that in combination with growth media in an agar gel is used to grow microorganisms).

As shown in Table 1, there are significant overlaps in equipment across lab types. As a general rule, the simplest and cheapest equipment and materials are likely to be encountered most frequently. These items include basic lab glassware, agar plates, simple incubators, reagents (i.e., compounds used and consumed during a chemical or biochemical reaction), and test animals. Some of this equipment, such as glassware, are general use items that alone do not provide useful insight about the nature of the laboratory activity. On the other hand, it will be rare to find complex and expensive laboratory equipment, such as a tissue

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	Laboratory Type	Common Equipment	Common Utility	Potential Legitimate Applications	Potential Illicit/Harmful Applications
Ι.	Home educational or hobby (page 3)	MicroscopeTest tubesGlass slidesReagents	Educational purposes	Simple biological experiments	N/A
11.	Beer or wine making (page 3)	 Simple fermentor (large jug, plastic container or specialized fermentation device) Beer ingredients such as barley, hops, malt, yeast Wine ingredients such as grape juice, yeast Bottles 	Making beer or wine	Not commonly used for other types of biological research	Suitable for growth of <i>Clostridium botulinum</i> bacteria, which produces botulinum toxin
.	Basic chemistry (page 5)	 Hot plate Glass beakers, flasks, test tubes Burner Chemical reagents Pipettors 	 Chemical assays Synthesis of chemical compounds 	 Biochemical assays Production of a chemical-based drug 	 Production of plant toxins: Ricin protein T-2 mycotoxin Production of chemical weapons
IV.	/. Biological Research Labs (pages 7–15)				
Α.	Basic microbiology (page 7)	 Flask shaker Incubator Flasks, beakers Pipettors Agar plates Growth media (dry or liquid) Autoclave Fermentor 	 Microbiology and bacterial growth for: DNA production Protein production General bacterial growth 	 DNA production (<i>E. coli</i> only) Production of a protein based drug (<i>E. coli</i> only) Testing antibiotics (miscellaneous bacteria) Developing yeast strains for beer or wine-making 	 Growth of bioterrorism bacterial agents: Anthrax (Bacillus anthracis) Plague (Yersinia pestis) Tularemia (Franciscella tularensis) Production of toxins: SEB (Staphylococcal Enterotoxin B) T-2 mycotoxin
В.	Protein production (page 9)	 Basic microbiology, plus: Separation columns Fermentor 	 Protein isolation Protein purification 	 Production of protein-based drugs Production of proteins used in biotech or diagnostic assays Antibody production 	 Production of bacterial toxins: Botulinum toxin protein SEB protein
C.	Molecular biology (page 10)	 Basic microbiology and chemistry, plus: Small and large plastic tubes Water bath Centrifuge Electrophoresis equipment 	 Biochemical assays Genetic engineering of DNA Genetic modification of bacteria 	 Developing biological or diagnostic assays Protein engineering Producing antibiotic resistant bacteria Protein isolation and production (early steps) 	 Genetically modifying dangerous pathogens: Antibiotic-resistant anthrax

Laboratory Type	Common Equipment	Common Utility	Potential Legitimate Applications	Potential Illicit/Harmful Applications
D. Tissue culture (page 12)	 Sterile flow hood Special incubators with CO₂ tanks Mammalian growth media Tissue culture dishes or flasks Autoclave 	Growth of human, animal and sometimes plant or insect cells	 Production of protein-based drugs Drug testing Growth of viruses Cell biology assays Plant propagation 	 Production of viral bioterrorism agents: Ebola virus Marburg virus Smallpox virus Flu (influenza virus)
E. Animal (page 12)	Caged animals, such as miceAnimal food	Assays for human or animal diseases	 Drug testing (chemical or protein-based) Virus infection for gene therapy Toxin testing 	Bioterrorism agent testingChemical agent testing

Table 1. An overview of laboratory types and their uses. (cont.)

culture hood (a specialized cabinet used in the growth of tissue or cells), in a residence or other out of the ordinary location. In many cases, equipment and materials may be improvised effectively, for example, substituting glass jars for beakers.

Although we present lab types individually in Table 1, it is common for multiple lab types to exist as part of a single lab set-up. Even when multiple lab types are part of a single set-up, the functionality of the equipment and materials largely remains the same. For example, bacterial growth media and agar plates are used to grow bacteria regardless of the set-up. The bacteria, in turn, may be used in molecular biology, protein production, and microbiology lab types.

I. Home Hobby and Educational Kits

Biological home hobby kits used for educational purposes (e.g., to supplement school curricula) or general interest are relatively common in a residential setting. Hobby kits usually provide supplies for simple procedures, such as basic bacterial or plant growth, and mostly have no expensive lab equipment, other than microscopes. Examples of common supplies in home hobby kits are shown in **Figure 1**. Home hobby kit components are, by themselves, insufficient for most nefarious use, but can provide some of the supplies needed for such activity. Labs with more expensive equipment or a large collection of chemical and biological reagents suggest a level of activity beyond an average home hobbyist.

II. Beer and Wine Making Set-Ups

Beer and wine making set-ups are other common biological lab types found in residential settings. Individuals involved in beer or wine making should have the basic, requisite supplies, such as grape juice, yeast, hops, and containers for fermentation as illustrated in **Figure 2**. While beer and wine making setups are not well-suited for biological research and development, they potentially could be used to grow *Clostridium botulinum* bacteria—grown in physical conditions similar to wine and beer production—for the end purpose of producing botulinum toxin, a highly toxic agent. Distinguishing observables include odors and the materials. For example, bacterial growth will have an unpleasant, rotting odor, in contrast to a fruity wine-like or yeasty beer-like odor commonly associated with wine and beer making, respectively. While individuals could grow *C. botulinum* in a beer or wine fermentation apparatus (which differ from fermentors typically used in biological research), *C. botulinum* production would require the use bacterial reagents, such as bacterial growth media; these are not used in beer or wine production. The presence of bacterial reagents and more advanced equipment in a space allegedly used only for producing beer or wine is suspicious.



Figure 1. Common supplies in home hobby kits. (Left) Microscopes and microscope supplies (which include slides and possibly stained biological materials on the slides) are the most common kit components. (Center) Some kits provide supplies for simple bacteria growth media, bottles of agar for making agar plates, empty plates, and pens for recording data and marking plates. (Right) Glassware and flasks containing bacterial growth media, some tubes with screw caps, and black rubber flask stoppers.



Figure 2. Beer and wine making supplies. Beer and wine is fermented in a variety of containers, including large buckets, jugs, or specialized metal containers as shown in the top row. Beer and wine making is accompanied using supplies shown in the bottom row, such as yeast, hops, and malt.

III. Basic Chemistry Lab

Equipment and materials found in a chemistry laboratory are designed for manipulating chemicals rather than biological agents. Common chemistry lab-type equipment is shown in **Figures 3–7**. Chemical-type laboratories likely are the most common illicit laboratories encountered in the field, most often used for drug synthesis; however, a chemical lab co-located with a biological lab probably is not for drug synthesis. The characteristics of a chemical lab can overlap with a molecular biology-type lab (see page 7), but there are differences. One significant difference is that many chemical reactions are performed at high temperatures (above 212°F, or 100°C) and require specialized heating elements. Chemical labs employ specialized apparatus for chemical separations, as shown in Figure 4; these are not typically found in a molecular biology lab. Additionally, chemical, such as ether, chloroform, benzene, potassium cyanide, aluminum hydride, or sodium nitrate among others.



Figure 3. Heating equipment commonly found in a chemistry-type laboratory. Chemical heating equipment generally heats to higher temperatures than found in biological labs. Note that a Bunsen burner, on the right, is also commonly found in biological labs, whereas the heating mantles to the left and center are not likely to be found in a purely biological lab.



Figure 4. Chemical separation equipment. Distillation equipment and other separation apparatus are commonly found in chemical labs, including illicit drug labs.



Figure 5. Chemical reagents found in chemical labs. Chemical reagents, like many biological reagents, are found in bottles and containers as shown above. However, the types of chemicals contained therein will differ when compared to biological reagents.



Figure 6. Glassware commonly found in chemical labs. General glassware, as shown above, also would be found in biological labs.



Figure 7. Pipettors and pipette tips commonly found in chemical labs. This equipment is used for working with very small volumes of liquids. Pipettors may also be found in biological labs.

IV. Biotechnology or Biological Research Labs

In this section we describe more advanced biological laboratory types that we collectively refer to as biotech or biological research labs. It is within this broad domain of lab types where distinguishing between one used for harmless or legitimate purposes and one used for harmful or illegitimate purposes becomes difficult due to significant material and equipment commonalities.

While less likely to be found in residential or other such settings than chemistry-type labs, there are reasons entrepreneurial scientists or other legitimate researchers may set up a biotech or biological research lab in an unconventional site. For example, a researcher may set up a home lab to establish control over the intellectual property generated by his/her efforts. The product or topic of research could be a drug to treat a disease, a more disease-resistant plant, a diagnostic tool, or even a process that helps in product development.

Importantly, however, the equipment and capabilities associated with these more advanced types of labs are those normally associated with the growth of harmful biological agents, to include bacterial agents, toxins, and viruses. (Due to a number of factors, we believe that terrorists and other criminals are less likely to attempt to grow viral agents than produce bacterial agents or toxins.) Any apparent activity involving the growth or presence of pathogenic bacteria in an unusual environment generally should be considered suspect and potentially dangerous to public health (see **sidebar**).

A. Lab with Microbiology Capabilities

A microbiology-type lab probably will be the most common true biological research or biotechnology lab encountered in a residential or other unordinary setting. Equipment and material common to this lab type are illustrated in **Figures 8–12**. In short, microbiology laboratories are typically used to grow bacteria—they require growth media and something to grow bacteria on or in (such as agar plates or a flask), equipment to control temperature, and equipment to sterilize materials used for bacteria growth. Most bacterial pathogens, including anthrax, plague, and other dangerous organisms, can be grown in a microbiology-type lab. Most dangerous bacteria cultivation visually will look no different from the growth of harmless strains. *C. botulinum* bacteria is an exception because it requires special growth conditions that lack oxygen.

Why E. Coli is Not Necessarily a Red Flag

The presence of or reference to the bacteria *Escherichia coli* (or *E. coli*) at a suspect location may or may not be indicative of malicious intent. On their own, certain strains of *E. coli* could be used as a bioterror agent because they cause sickness in humans, most notably food poisoning. On the other hand, *E. coli* is a very commonly used tool in microbiological development. For example, *E. coli* is used to grow DNA, produce proteins, and in genetically engineering genes. (Growth of other types of bacteria for these legitimate purposes is unlikely, except when testing antibiotics.) In its role as a tool rather than an end product, *E. coli* can be used when developing other dangerous pathogens. For example, creating antibiotic-resistant anthrax uses genetic engineering techniques that require an antibiotic resistance gene that would be grown in *E. coli*.



Figure 8. Bacterial growth media reagents, including liquid media in a bottle and prepared media in flasks (with unprepared dried media in foreground). A stack of agar plates are shown, with the red agar plate showing bacterial growth on the surface of the plate. Bacteria grown in liquid media will be a cloudy mixture, whereas media without bacteria will be clear. Bacteria on a plate will appear as light spots or streaks, whereas agar plates without growth will appear as a very smooth surface without variation in color.



Figure 9. Incubators used to control the temperature during bacterial growth. Some incubators can include a shaking ability, which helps with bacteria growth. Both agar plates and flasks would be placed in incubators.



Figure 10. Shakers and water baths are used to grow bacteria. Water baths can be used like incubators to control temperature, and shakers help provide air to bacteria for best growth. This equipment may be commonly found in a residential lab.



Figure 11. Autoclaves and a pressure cooker used for sterilization of growth media and equipment. Materials used in bacterial growth must be sterilized in either an autoclave, pressure cooker, or even a household oven. This equipment may be found in a residential lab.



Figure 12. Fermentors for large-scale bacterial growth. These devices are used when large quantities of bacteria are desired. This equipment is expensive and requires some technical skill to operate. Thus, these devices are less likely to be encountered in a residential setting.

B. Lab with Protein Research Capabilities

Protein laboratories are used for producing and purifying proteins, and are less likely to be encountered in a residential setting or other unordinary location. Terrorists or other criminals could produce proteinbased toxins, such as botulinum toxin or Staphylococcus Enterotoxin B (SEB), in a protein laboratory, but sophisticated purification often is not required for use of the biological agent in an attack. However, non-terrorist related, illicit biological activity might include botulinum toxin production for cosmetic purposes, in which case specialized purification is required. Specific equipment used in protein purification is shown in **Figures 13** and **14**.



Figure 13. Protein purification columns used to produce pure proteins. Columns can be small in size ~ 1 inch (left) or 12 inches or more (right).



Figure 14. Liquid chromatography equipment for protein purification. Liquid chromatography equipment is less likely to be encountered in a residential lab due to expense and the high level of technical skill required for operation.

C. Lab with Molecular Biology Capabilities

Molecular biology labs are used for biochemical experiments, genetic engineering, genetic modification of cells, and the initial steps towards producing proteins. Some of these processes, such as gene manipulation, might be used when modifying dangerous bacteria. For example, molecular biology labs can be used as part of the process of making antibiotic-resistant anthrax bacteria. Equipment common to this laboratory type is shown in **Figures 15–20**. Of note, the equipment and materials used therein can overlap with that used in a chemical lab, but differ in key ways. For example, molecular biology is conducted at temperatures up to 212°F (or 100°C, the boiling temperature of water) and does not typically require equipment for high heat applications. Molecular biology labs frequently use biological molecules in biochemical reactions, these molecules include DNA, antibodies, enzymes, or proteins. These materials are typically stored in a refrigerator or freezer and the names are found on the side of the tube, with the tube often only an inch in size. These reagents are typically not used in a chemistry lab.



Figure 15. Pipettors and pipette tips are commonly found in molecular biology labs, as well as chemical-type labs. This equipment is used for working with very small volumes of liquids.



Figure 16. Centrifuges are commonly found in molecular biology type labs and can be found in chemical-type labs. Centrifuges vary widely in size, but all are designed to spin tubes or other containers.



Figure 17. Tubes, beakers, and other containers used to hold liquid solutions are commonly found in molecular biology type labs and most other lab types. These are general usage items.



Figure 18. Shakers and water baths are commonly used in molecular biology type labs to regulate the temperature of reagents and biochemical reactions. These items are uncommon in chemical labs.



Figure 19. Molecular biology kits are very common in molecular biology-type labs. The kits shown above have a similar appearance, but the labels indicate (from left to right) a DNA purification kit, a polymerase chain reaction (PCR) kit, and a kit for mutagenesis. These kits contain enzymes and reagents that are a key way to distinguish a chemistry lab from a molecular biology lab. Most enzymes would be found in tubes stored in a refrigerator or freezer. Examples of enzymes include restriction enzymes, ligase, polymerase, kinase, phosphatase, and reverse transcriptase. These terms normally are located on the side of enzyme-containing tube.



Figure 20. Electrophoresis is commonly used in molecular biology-type labs to separate DNA, RNA, and proteins. The equipment shown includes the electrophoresis apparatus and the electrical power supply required for operation.

D. Lab with Tissue Culture Capabilities

Tissue culture laboratories are used to grow non-bacterial cells, such as human cells, animal cells, plant cells, or even insect cells. Tissue culture is required for most research on cells that are not bacteria. This lab type is probably the least likely to be encountered in a residential setting, because tissue culture labs require very expensive specialized equipment and significant laboratory space. **Figures 21–23** illustrate some of this material and equipment.

E. Lab with Animal Research Capabilities

Animal laboratories, are likely to be associated with another type of laboratory set-up, such as a microbiological or chemical-type lab, rather than to be found by themselves. The presence of animal lab-type capabilities suggests testing of a drug or other product, usually in the late stages of development. A terrorist or other criminal could use such a set-up to test harmful biological agents. The materials and animals used in an animal lab are easily acquired and inexpensive. **Figure 24** shows some of the materials needed for an animal lab, but could also include any sort of improvised animal handling equipment. Animals used in biological testing could be mice, rats, hamsters, gerbils, guinea pigs, rabbits, cats, or dogs.



Figure 21. Tissue culture containment hoods for sterile handling of non-bacterial cells. This equipment is costly and requires a considerable space for operation.



Figure 22. Tissue culture incubators are required for the growth of non-bacterial cells. Tissue culture incubators require carbon dioxide gas for operation (which is not required for bacterial incubators).



Figure 23. Tissue culture supplies include specialized media, serum, and flasks not used to grow bacteria, although they make look similar. The picture on the far left shows the red-colored "buffer" and some additives. Calf serum (brown in color) is shown center, and flasks and dishes on the right. Cells grown in tissue culture will not look as cloudy as bacterial growth. It is possible to have tissue culture cells growing in the media, yet the media will appear nearly clear. In some cases the cells will be stuck to the side of the flask or dish.



Figure 24. Basic supplies for an animal lab include some kind of cage, bedding material, animal food, and the animals themselves. All of these items are found in a pet store.