CHAPTER 6

Laboratory Best Practices

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Preface

The laboratory holds a unique role in healthcare outbreak response, providing key information to help initiate and guide investigations. Whereas in previous chapters we introduced and described some basic concepts regarding the role of laboratory partners, here we present more detailed explanations, examples, and considerations, with an emphasis on best practices.

6.0 Introduction

The role of the laboratory in healthcare-associated infection (HAI) outbreak response is critical, beginning with organism identification and routine antimicrobial susceptibilities. Given the availability of advanced technologies, communications, and networks, a laboratory may be able to provide information regarding novel resistance patterns and mechanisms, identify clusters of related illness, and generate data to be used by public health and healthcare partners to detect and respond to outbreaks.

Public health laboratories (PHLs) are required to notify public health authorities upon the identification of reportable diseases. PHLs are also well positioned for the early recognition of sentinel cases (those involving unusual pathogens or resistance patterns) or clusters. Additionally, PHLs are encouraged to promptly alert epidemiology partners after receiving a request from a healthcare facility or provider to perform typing of multiple isolates for an apparent cluster or outbreak.

Many aspects of outbreak response benefit from active collaboration and coordination between the PHL and other public health and healthcare partners. Examples include clarifying requirements and streamlining procedures for the reporting of potential outbreaks and the retention/submission of specimens and/or isolates by commercial, private, and academic laboratories—both in state and out of state (incorporating these into guidance or administrative codes). PHLs also may serve a key function in the support of outbreak response activities by developing and maintaining an inventory of specialized testing and characterization services available in house or in other laboratories and by providing guidance to partners regarding how to access these services.

This chapter begins with an overview of the various types of laboratories and their roles, followed by a description of laboratory functions that support outbreak response and the importance of reliable and clearly communicated data. For laboratory data to be meaningful and useful, they must be accurate, timely, of high quality, and presented

in a clear and concise manner. Specific to laboratorians, we also address safety practices to be followed when working with antimicrobial-resistant (AR) pathogens and the validation of AR and HAI test methods.

6.1 Types of Laboratories and Roles

6.1.1 Public Health Laboratories

At least one state public health laboratory is located in each state in the US; additional governmental laboratories are often found in large cities or counties. Despite diversity in discipline and range of capability, these laboratories are dedicated to promoting and protecting the health of citizens. As the national public health laboratory, the Centers for Disease Control and Prevention (CDC) offers a wide scope of testing, guidance, research, and development services.

In 2016, CDC established the Antimicrobial Resistance Laboratory Network (AR Lab Network), which serves to detect and characterize AR pathogens and communicate findings and resources to prevent infection. The seven AR Lab Network regional laboratories offer access to a wide variety of specialized testing including colonization testing, identification of resistance mechanisms, specialized susceptibility testing using reference methods, and next generation sequencing (NGS). Although some of these testing services may also be available at state or local public health laboratories, reference laboratories, or large clinical laboratories, the regional laboratories assure a centralized mechanism to access this testing for all facilities.

The national, non-profit professional organization dedicated to strengthening public health laboratory systems is the Association of Public Health Laboratories (APHL). As a representative of national, state, and local governmental health laboratories, APHL is positioned to capitalize on the available diversity in PHLs, foster communication, provide expert-derived guidance, and work with federal agencies to develop and execute national health initiatives such as those related to HAIs and AR pathogens. Related toolkits, guidance documents, offers of training opportunities, and various other resources are available at www.aphl.org.

6.1.2 Clinical Laboratories

Clinical laboratories, often based in hospitals, provide a wide range of laboratory procedures that aid clinicians in the diagnosis, treatment, and management of patients. Commercial laboratories, some of them quite large and national in scope, provide similar functions. Clinical laboratories serve an integral role in the detection and characterization of a wide array of HAIs and AR pathogens. More complex analyses of pathogens such as *Mycobacterium tuberculosis* complex (MTBC) and *Candida* spp., however, may require isolates to be transferred to a commercial or reference laboratory, or a state PHL.

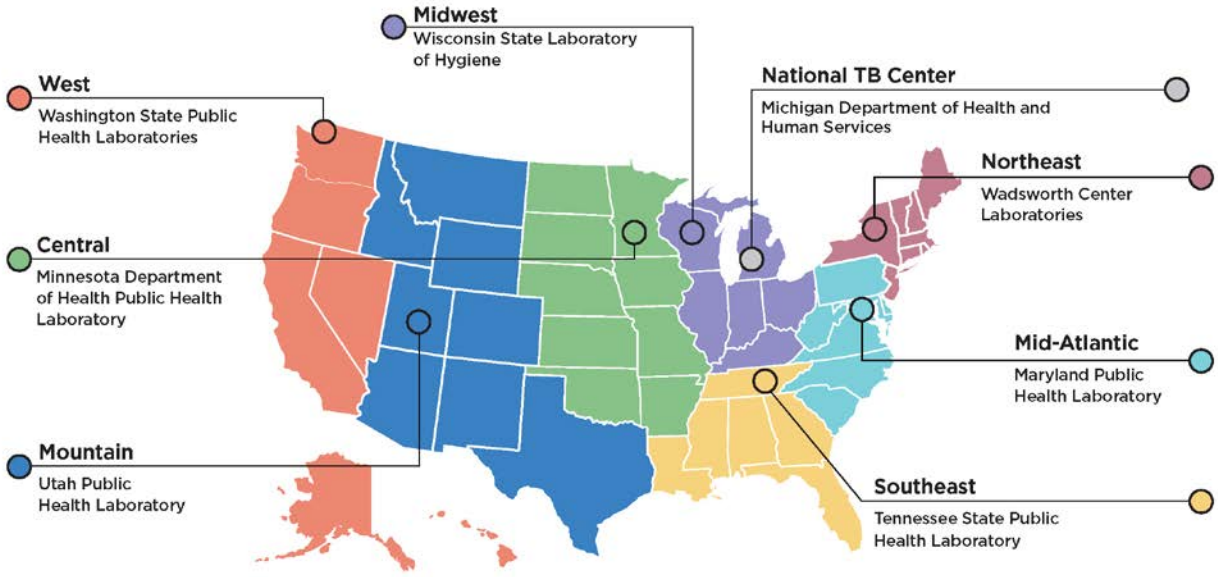
Antimicrobial susceptibility testing services in clinical laboratories may include growth and molecular-based analyses of some of the more common Gram-positive and Gram-negative bacteria. Clinical laboratory staff should be knowledgeable of applicable surveillance and reportable disease regulations or guidance material and consider these when deciding to proceed with AR testing.

6.1.3 Reference Laboratories

Reference laboratories may offer extensive and specialized testing to support surveillance activities. These facilities may be independent laboratories or associated with public health agencies or educational or research institutions. The same considerations described in the previous section regarding jurisdictional reporting requirements apply to reference laboratories.

In addition to its function as the US national reference laboratory, CDC established and supports the AR Lab Network (described in section 6.1.), greatly expanding the capacity of public health facilities to detect and respond to AR cases and outbreaks. The Network consists of laboratories in 50 states, four cities, and Puerto Rico, and includes seven regional laboratories and the National Tuberculosis Molecular Surveillance Center (Figure 6.1).[1] The Network aids the public health community in the quick detection of emerging AR threats in healthcare, food, and the community; rapid response at the state and local level to contain pathogen transmission; and increased understanding of emerging AR threats.[1]

Figure 6.1 | Antimicrobial Resistance Laboratory Network Map of Regional Laboratories[1]



The AR Lab Network assists each local jurisdiction with AR pathogen surveillance, but the Network as a whole functions as a surveillance entity with the capacity to provide information on national trends and to detect outbreaks. When state or local laboratories have neither the capability nor the capacity, the Network's regional laboratories can provide additional testing. At the time of this writing, this includes advanced testing for *Acinetobacter, Aspergillus fumigatus, Candida auris,* carbapenem-resistant Enterobacterales (CRE), colistin resistance among extended-spectrum beta-lactamase (ESBL)–producing organisms, *Mycobacterium tuberculosis, Neisseria gonorrhoeae, and Streptococcus pneumoniae*. Regional laboratories that detect organisms and mechanisms of resistance of public health significance routinely alert public health partners to trigger investigations and other actions to prevent transmission.

6.2 Laboratory Functions in Support of Healthcare Outbreak Response

6.2.1 Surveillance

Surveillance, as it relates to HAIs, involves collecting and analyzing health-related data to evaluate the quality of healthcare that is being provided, identifying opportunities for improvement and monitoring progress following intervention. Laboratories are integral to the surveillance process, as they generate, analyze, and submit data to surveillance programs, and may be the first healthcare partner to identify an unusual occurrence or frequency in their results. Laboratories serve as the first level of action in the surveillance process, and therefore, their staff should be cognizant of how, when, and to whom data can be shared to be most impactful.

Hospitals and clinical laboratories monitor and report certain drug-resistant organisms and HAIs to meet a variety of different regulatory requirements. The Centers for Medicare & Medicaid Services (CMS) mandates the reporting of certain HAIs through the National Healthcare Safety Network (NHSN).[2] States and counties may require that hospitals report certain pathogens, diagnoses, and/or multidrug-resistant organisms (MDROs). In addition, CDC provides guidance for the initial response to a novel or targeted MDRO or resistance mechanism. Such a response may involve a combination of prospective and retrospective laboratory surveillance, depending on the resistance pattern of interest. More information on surveillance, including reportable and notifiable diseases, is provided in Chapter 2.

6.2.2 HAI and AR Pathogen Detection and Confirmation

As described in Chapter 5, section 5.1.2, early detection of the causative agent is critical to appropriate treatment and the prevention of additional cases. The laboratory has numerous assays on hand to support the identification and confirmation of HAI and AR pathogen cases and to subsequently assist with the diagnostic aspects of these case definitions where needed. Tests involving the physical characteristics of a microorganism are known as phenotypic or growth-based (e.g., culture), whereas tests involving genetic properties are called genotypic or molecular-based (e.g., polymerase chain reaction [PCR] or sequencing). With regard to the detection and confirmation of new and emerging AR pathogens, each type of test displays advantages and limitations (Table 6.1).

Table 6.1 | Phenotypic and Genotypic Tests

TEST TYPE	METHOD, OUTPUT	EXAMPLES	ADVANTAGE	LIMITATION
Phenotypic	Zone of inhibition, Millimeters	Kirby-Bauer disk diffusion susceptibility test	• Simple to perform • Applicable to several antibiotics • Applicable for diverse organisms (e.g., *Haemophilus influenzae, H. parainfluenzae, Neisseria gonorrheae, and N. meningitides*)[7] • Standardized method • Cost-effective • Results correlate to known resistance/ susceptibility based on defined breakpoints for known resistance	• Detection may be limited to the growth rate of the organism (takes 16–24 hours for results) • May require a pure culture of an actively growing organism • Visual/manual data interpretation requires expertise and competency • Breakpoints are not defined for all organism/drug combinations
Phenotypic	MIC, reported concentration, µg/mL	Automated: Vitek®, MicroScan™, Sensititre™, Phoenix™ Manual/semi-automated: gradient strips (e.g., ETEST® or MTS™ strips), broth dilution, agar dilution	• Simple to perform if using an automated method • Applicable to several antibiotics • Applicable for diverse organisms (e.g., *Haemophilus influenzae, H. parainfluenzae, Neisseria gonorrhoeae, N. meningitidis*)[8] • Standardized method • Cost-effective	• Detection is limited to the growth rate of the organism (takes 16–24 hours for results) • May require a pure culture of an actively growing organism • Visual/manual data interpretation requires technical expertise and competency • High volume of reagents • Requires multiple dilutions • Requires expertise

Table 6.1 | Phenotypic and Genotypic Tests

TEST TYPE	METHOD, OUTPUT	EXAMPLES	ADVANTAGE	LIMITATION
Genotypic	PCR	Lab-developed tests, CDC-developed tests, Commercial platforms	• Can be culture-independent • Rapid • Sensitive • Specific • Detection of multiple targets simultaneously • High throughput	• Presence of resistance genes or mechanisms does not always confer phenotypic resistance • If performed without prior culture, there is no isolate for further investigation • Inability to distinguish viable from nonviable organism • Advanced technical skills may be required for some assays • High instrument and consumable costs • Risk of amplicon contamination
Genotypic	Nucleic acid sequencing	Targeted sequencing, NGS, Long read, Short read	• Novel resistance mechanisms can be detected • Novel pathogens may be detected • Identifies genetic relatedness among isolates • Mutations (e.g., single nucleotide polymorphisms [SNPs]) that confer new resistance or altered resistance patterns may be detected • Can be used to resolve discrepancies in other test results (e.g., mCIM+ / PCR-)	• Gene target associated with resistance must be known • May require pure culture of actively growing organism • Detection is limited to the processing time of sequencing and analysis, which can be time consuming • High technical skill is required • High instrument and consumable costs • Increased potential for cross-contamination (can be identified in analysis through pipelines) • Inability to distinguish between viable and nonviable organism • Presence of target does not always confer phenotypic resistance but may be relevant for clinical management; infectious disease consult may be warranted • New targets require additional validation

6.2.2.1 Phenotypic Testing

The emergence of matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) greatly improved the ability of laboratories to identify organisms rapidly and efficiently down to the species level. This has contributed to the reporting of organisms with less familiar nomenclature, such as a unique species previously characterized as part of a group of organisms or a complex. For example, MALDI-TOF MS can be used to identify *Enterobacter asburiae*, which otherwise would have been labeled *Enterobacter cloacae* complex when using traditional biochemical tests.

Similarly, enhanced characterization of bacterial and fungal species through molecular techniques such as DNA sequencing has prompted reclassification or renaming of some species. The laboratory can be helpful in assisting infection preventionists and epidemiologists in navigating these changes in nomenclature, particularly when including former microbial names in case findings (e.g., the 2017 reclassification of *Enterobacter aerogenes* to *Klebsiella aerogenes*).[3] When relying on laboratories that identify organisms by applying more traditional methods, such as biochemical tests (e.g., API 20E), or use of older automated instruments with outdated software, it is important to bear in mind that discrepancies may occur when the organism identification is confirmed using newer technologies or more up-to-date software.

Phenotypic antimicrobial susceptibility testing (AST) describes conventional methods that establish antibiotic resistance or susceptibility by measuring growth (or lack thereof) of an organism in the presence of a drug. To interpret the results, phenotypic testing methods require that the organism be identified and grown in a pure culture. Several manual and automated tests are available, including disk diffusion, agar dilution, broth microdilution, broth dilution, and gradient strip diffusion. The Kirby-Bauer disk diffusion susceptibility test results in a zone of inhibition around a disk containing antibiotics of known concentration. The size of the zone correlates to the susceptibility or resistance of the organism to the drug and is inversely proportional to the minimum inhibitory concentration (MIC). Zone size alone is meaningless and should not be reported to clinical providers.[4] The MIC is the minimum concentration of antibiotic necessary to inhibit growth. It can be determined by both microdilution and the ETEST®. It can also be referred to as the minimum bacteriostatic concentration because growth is inhibited but the organism is not killed. In contrast to the MIC, the minimum bactericidal concentration (MBC) is the minimum concentration necessary to kill the organism. Both the MIC and zone sizes can be interpreted to be resistant, susceptible, or susceptible dose-dependent results based on breakpoints defined in the *Clinical & Laboratory Standards Institute (CLSI) M100*.[5]

Regardless of the test selected, laboratories should use the current interpretive breakpoints published by organizations that develop standards, such as CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These sources will have the most up-to-date recommendations for breakpoints and detection strategies. Often, Food and Drug Administration (FDA)–cleared products may not reflect current breakpoints and, therefore, validation studies may be necessary. Validation studies are also warranted when laboratory-developed tests (LDTs) or other methods selected have not been approved by the FDA, such as those labeled “for research use only (RUO),” which are not intended for use in patient diagnostics. Laboratories should be aware of the new College of American Pathologists (CAP) requirement[6] that all breakpoints should be identified and recorded, and that any breakpoints updated prior to 2021 must be current as of January 1, 2024. APHL and the AR Laboratory working group have developed a toolkit to assist laboratories in this transition.

6.2.2.2 Genotypic Testing

Molecular methods may be used to predict antibiotic resistance in vivo through the detection of specific genetic targets or mutations. Identification of a gene target or mutation may be useful in predicting antibiotic resistance in vivo. The primary benefit of molecular antimicrobial susceptibility tests (ASTs) is that they allow direct testing of clinical or environmental specimens without the need for culturing. When applied in this manner, genotypic ASTs are more rapid than phenotypic methods. However, these systems lack the ability to distinguish between viable and nonviable organisms, and genetic indicators of resistance do not always confer resistance phenotypically.

The intended use of the assay, whether for screening or identification, must be considered, because this dictates how the results are reported and the data are interpreted. Screening tests typically exhibit high sensitivity and low-to-moderate specificity since they are designed to quickly assess a specimen for the presence or absence of the target. Such tests allow for a presumptive result and should be reflexed to culture to isolate and identify the organism. Alternatively, identification tests usually possess characteristics of high sensitivity and specificity, and therefore are more accurate. Depending on the assay, additional testing may be necessary before reporting a confirmed result. Discerning a presumptive from confirmatory result is critical when reporting data to epidemiologists and other partners. Nevertheless, in many cases preventive action can still take place based on a presumptive or preliminary result to reduce the risk of transmission. Confirmed and final results should be reported as soon as they are available.

6.2.2.3 Next Generation Sequencing

During the past two years, advancements in next generation sequencing (NGS) technology have led to the use of NGS not only for identification purposes but also for the detection of drug-resistant markers. NGS can play an important role in HAI outbreak investigations, including those involving MDROs.

Currently, this technology may be cost-prohibitive due to the high upfront cost of equipment and the need for highly specialized bioinformaticians. In the near future, however, NGS equipment is expected to become affordable and trained personnel more widely available.

NGS is relevant and useful to antimicrobial resistance surveillance in two distinct ways. The first is in the detection of novel resistance genes that may not be detected using current molecular (PCR) assays. This is illustrated in a recent case of *Pseudomonas aeruginosa* infection. The organism proved to be nonsusceptible to most antibiotics evaluated, was deemed positive for carbapenemase using the modified carbapenemase inactivation method (mCIM), and was found negative for all PCR targets for which it was tested. NGS analysis detected the presence of the $bla_{\text{sim-1}}$ gene, which is the first time this target was detected in the US.[7]

The second use for NGS among antimicrobial resistance surveillance is to determine the relatedness between pathogen strains. This is particularly relevant to assess transmission within or between healthcare facilities. Strains that are highly related to one another are more likely to share a common source.

6.2.2.4 Terminology

Laboratories should remain current with the accepted definitions for various MDROs that are resistant either to a primary antimicrobial drug or to one or more drugs from different drug classes. Some common or targeted MDROs described by CDC are listed in Table 6.2.[9]

Table 6.2 | Common or Targeted MDROs

ORGANISM	DRUG RESISTANCE
Staphylococcus aureus	Methicillin-resistant *S. aureus* (MRSA) Vancomycin-intermediate *S. aureus* (VISA) Vancomycin-resistant *S. aureus* (VRSA)
Enterococci	Vancomycin-resistant Enterococci (VRE)
Escherichia coli *Klebsiella*	Extended spectrum cephalosporin-resistant
Proteus mirabilis	Extended spectrum cephalosporin-resistant *ampC* phenotype
Enterobacterales	Carbapenem-resistant Enterobacterales (CRE)
Pseudomonas aeruginosa	Carbapenem-resistant *P. aeruginosa* (CRPA)
Acinetobacter	Carbapenem-resistant *Acinetobacter* (CRAB)

No single list of MDROs is comprehensive, but standard terminology applies throughout.[5]

- Susceptible (S) indicates growth is inhibited by drug treatment.
- Intermediate (I) indicates growth is inhibited by a drug dose higher than that required by a susceptible MDRO.
- Resistant (R) indicates growth is not inhibited by treatment with at least one drug.

- Multidrug resistant (MDR) indicates acquired resistance; “not susceptible” to at least one drug in three or more drug classes.
- Extensively drug resistant (XDR) indicates acquired resistance; “not susceptible” to almost all drug classes but sensitive to at least one drug class.
- Pan-drug resistant (PDR) indicates acquired resistance to all drugs available.

6.2.2.5 Saving Specimens and Isolates

During an outbreak investigation, all relevant organism isolates should be retained by the clinical laboratory, PHL, or reference laboratory to ensure availability for strain typing. In the event culture-independent diagnostic tests (CIDTs) are used and an isolate is unavailable, laboratories should send CIDT-positive samples to the PHL within 24 hours after the positive result has been obtained. Clinical laboratories should coordinate with PHL staff prior to shipping. For circumstances outside outbreak management, laboratories should work with the infection prevention team to develop a routine laboratory policy for saving isolates. The policy should define which isolates are retained and for how long, and should also address the retention of original specimens, their derivatives, and any specimens with uncommon results.[10] Such a retention policy is valuable: specimens can be retained for repeated or additional testing when needed, further investigation for public health purposes, quality control purposes, and new test validation. Extended storage (up to 10 years) is ideal for specimens and isolates exhibiting unusual, emerging, and novel resistance mechanisms. An inventory system covering retained specimens and isolates should be in place for the biosafety and biosecurity of the laboratory. The laboratory must consider the needs of the patient, the storage capacity of the laboratory, and future test development.

6.2.2.6 Characterization Testing

Considerations and best practices for establishing a case definition and managing case findings are discussed in Chapter 5, section 5.1.6. This section provides information regarding laboratory testing that may be used to support an outbreak investigation through characterization and relatedness testing.

An outbreak response may require laboratory support beyond that associated with typical clinical specimens. Each clinical laboratory needs to be able to rapidly identify AR pathogens for subsequent referral to a PHL or reference laboratory for full characterization. Timely communication and collaboration between laboratories are critical. Outbreak investigation and response may include surveillance activities such as point prevalence surveys and admission screening, which can require substantial laboratory resources. These can involve processing a large number of samples using methods not routinely performed in that laboratory. They may require healthcare personnel testing or environmental testing if personnel or an environmental reservoir is potentially implicated in the outbreak during the investigation. During an investigation, it may be appropriate to perform molecular analyses such as PCR and NGS to identify mechanisms of resistance and to determine genetic relatedness between clinical isolates and/or environmental sources.

If it is determined through NGS that two or more organisms are genetically related, it is likely that they share a common source. This could be evidence of patient-to-patient transmission or a common reservoir of infection. Species identification and susceptibility results may provide evidence for or against an epidemiologic link. However, because many organisms have predictable resistance patterns, susceptibility patterns are not sufficiently discriminatory and additional tests are required. Thus, genotypic or DNA-based typing methods have replaced phenotypic typing methods that discriminate poorly among isolates. Given the dramatic reduction in cost and time needed to sequence a bacterial or viral genome, NGS has now become the gold standard for molecular typing of healthcare-associated pathogens and has largely replaced older genotypic methods such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). If a laboratory cannot perform strain typing when it is deemed necessary, isolates can be sent to the PHL for testing.

6.2.3 Reporting to Epidemiology and Other Partners

Detection of clusters and possible outbreaks can originate from a variety of sources, as described in Chapter 4. As epidemiology staff gather information, they rely on laboratory results to provide meaningful details relevant to a possible event. Thus, the laboratory plays a key role in outbreak detection through the generation of testing results and compilation of these results into reports. Laboratory testing should be performed accurately and in a timely manner, with reports made available upon completion. Laboratory results are crucial in identifying the true cases associated with an event. Data must be reported in a clear and concise manner so that it may be evaluated without interpretation biases, as is possible when technical details are provided without proper context or guidance.

Reports such as antibiograms, which include antimicrobial resistance surveillance data for a defined population, may be shared with epidemiologists, infection control practitioners, clinicians, and other stakeholders. Within a facility, antibiograms may be developed for specific areas such as an intensive care unit or infectious disease unit. Clinical laboratories should provide periodic summaries of selected microbiology results, such as antibiograms specific to HAI pathogens or trends in selected AR pathogen incidence over time. Hospital laboratory personnel may need to call infection prevention program personnel directly to report some results to ensure that timely control measures are implemented (e.g., transmission-based isolation precautions and prophylaxis of contacts). The list of results that require such urgent test reporting may vary based on federal, state, or local regulations and on requests or requirements from the facility; however, some examples of organisms requiring immediate notification follow:

- *Neisseria meningitidis* from a sterile site
- *Legionella*
- *Mycobacterium tuberculosis* (or a positive result from an acid-fast bacillus test of respiratory samples)
- Potential agents of bioterrorism (e.g., *Bacillus anthracis* or *Yersinia pestis*)
  - Note: If presence of a potential agent of bioterrorism cannot be ruled out in the laboratory, it is important to reduce access to the primary specimen or cultured isolate, and to contact the state or local PHL immediately.
- AR pathogens (e.g., carbapenem-resistant Enterobacterales, vancomycin-resistant *Staphylococcus aureus*, and *Candida auris*)

Epidemiologists and infection preventionists may be able to use these reports to support an investigation regarding the source and spread of disease within a facility. They may also collaborate with other partners to support the development of guidelines to prevent future outbreaks and reduce the incidence of antimicrobial resistance. It is important to establish and maintain good working relationships with partners in epidemiology and HAI programs, hospital infectious diseases and infection control departments, and microbiology laboratory directors. One way to do that is to establish a committee that meets two to three times each year. More information on communication among partners can be found in Chapter 5, section 5.1.3.3.

Reporting procedures must allow for the timely transmission of laboratory results to infection prevention personnel and relevant state and local reporting systems. Because different facilities often use highly variable methods for storing and tracking data, it is essential to allow for reliable data exchange so that relevant information is not lost during transmission. It is also beneficial to allow various options for reporting to be available. These options can include secure transmission via legacy systems such as fax and telephone as well as electronic submission such as secure email and electronic laboratory reporting (ELR).

In addition to the modes of reporting given above, hospital laboratory staff should meet regularly with infection prevention personnel to ensure that communication channels are direct and effective, and to discuss areas of mutual concern such as the status of all ongoing cluster or outbreak investigations. Together they can also determine whether supplementary testing, such as organism typing or environmental cultures, will be necessary. It may prove beneficial to bring in state and local public health partners as well.

Ensuring that the aforementioned reporting mechanisms are in place may be challenging if a hospital has outsourced laboratory services (such as to a commercial laboratory or a central laboratory within a large healthcare system), but reporting remains necessary to provide optimal HAI and AR pathogen outbreak detection and response.

6.2.4 Detection of HAI Outbreaks by the Laboratory

Chapter 4 established that detection of an HAI outbreak can occur at any level, but here we explore how the laboratory can support detection. Essentially, laboratories provide support through characterization testing, which may be used to guide outbreak response and monitor developments. The use of PHLs and the AR Lab Network regional laboratories can provide the necessary structured framework for improved communication, coordination, and tracking during an HAI outbreak.

Characterization of isolates may be performed to assist with identifying the source of an outbreak and to link clinical cases and/or environmental sources; however, data resulting from such analyses may be complex and require interpretation. Next generation sequencing is commonly used to investigate isolates at the genetic level and yields large amounts of data requiring subsequent analyses with sophisticated software programs. Multiple sequences can then be further examined to determine genetic relatedness, which is depicted using a phylogenetic tree. When data from multiple patients or sources are compiled and reported in such a manner, a description should be included to clearly indicate which isolates are and are not likely to be genetically related. These data, along with other epidemiologic findings, may be used to define the scope of the outbreak, the attributed source, and risk factors, or to otherwise link cases based on common features. For this to be successful, communication among partners in a timely manner is essential.

HAI outbreaks are defined by an increase in the number of cases of infections among patients or staff above the expected number of cases; this increase can be determined through ongoing surveillance. Pathogen-specific surveillance can be used to monitor select pathogens through reporting by healthcare providers and laboratorians, and should consider inclusion of information on patient exposure, risk, and underlying conditions. The full spectrum of specific pathogens under surveillance may be determined by infection prevention and control units within healthcare settings.

Pathogens may be reportable beyond the original facility, and this may require submission of a specimen from the laboratory serving the healthcare facility to an appropriate local or state public health laboratory. Notification to the CDC is required for nationally notifiable pathogens or for select reporting programs. Specimen submission to the CDC or an AR Lab Network regional laboratory may be a requirement or necessary when additional testing is requested.

As cases are identified and reported, a response could occur at multiple levels, beginning first with the infection prevention team at the healthcare facility, then followed by public health epidemiologists working closest with the reporting laboratory. A first response effort would include collection of additional follow-up data to help identify how acquisition or transmission occurred. These data can be used to link cases based on relevant findings. Specific metadata for each isolate are invaluable for epidemiologic study and could include basic details about the specimen (such as collection date, source, submitting facility, and test results), patient information (e.g., age, sex/gender, and residence), and patients' significant risk factors (e.g., comorbidities, recent travel, unique exposures, or behaviors).

Concurrent review of microbiology data remains the most common HAI and AR pathogen case-finding method used by hospital infection prevention programs, and requires prompt, accurate, and reliable reporting of positive laboratory test results. This communication may occur in a number of ways, but most hospital infection prevention programs have in place electronic surveillance systems that interface directly with the laboratory information system (LIS) or electronic medical record (EMR) system. Such electronic surveillance systems allow infection prevention teams to configure alerts and efficiently monitor test results in real time.

Detection may also occur at the local or state health department through regular systematic review of routine surveillance data, review of patient reports, or review

of reports from alert healthcare personnel. When an outbreak is identified at the public health level, an outbreak number is often assigned; this allows all related communications, laboratory findings, and reports to be connected. With adequate staff and expertise, the local health department can initiate and coordinate the responsibilities of the investigation to determine who will lead the response and what is needed from participating laboratories. If local public health capabilities are insufficient, the state health department will lead the response. Details such as laboratory testing methods and the facility at which testing will be performed, the timeframe of the investigation, and resources are agreed upon to effectively manage the investigation. Given the logistical challenges with analyzing large datasets, having electronic accessioning systems in place will result in a seamless linkage of isolate test results to epidemiology data, while allowing for additional laboratory or epidemiology data to be added.

Whether an increase in the number of cases is detected by healthcare personnel or the laboratory, public health officials and infection preventionists should be contacted to coordinate specimen submission and initiate the formal chain of reporting. Public health officials should also collaborate with healthcare personnel to assist the facility with coordination of effective control measures as well as additional specimen collection if further testing or confirmation is needed. If the laboratory providing testing is located offsite from the healthcare facility, enhanced coordination with the facility and health department may be needed in response to the greater logistical challenges associated with specimen collection, transport, testing, and data transmission between different systems.

To ensure the swift detection of outbreaks, effective communication of test results between the laboratory and the infection prevention program is key. In particular, electronic systems that communicate laboratory results to the infection prevention team in real time may help identify outbreaks as they happen. It is important to note, however, that concerns about a cluster or an outbreak are sometimes first raised by an astute laboratory technologist, nurse, or other member of the healthcare team. Outbreak detection should therefore be a multidisciplinary effort that encourages all personnel to report concerning nosocomial infections to the infection prevention program.

6.2.5 Environmental Testing

Environmental testing is an attractive addition to outbreak investigations because it can test hypotheses about transmission, identify pathogen reservoir(s) and later evaluate the efficacy of interventions. Environmental testing is generally not encouraged, however, except in circumstances in which an environmental source has been implicated or the literature supports environmental testing. In addition, it should be undertaken only after consulting with an epidemiologist experienced in outbreak investigations. Many clinical microbiology laboratories do not possess expertise in testing environmental samples, and most do not validate their existing tests for use on nonhuman specimens. When there is limited capacity in the laboratory to perform such testing, specimens should be referred either to laboratories that specialize in environmental microbiology or to the jurisdictional public health laboratory. Some PHLs may include environmental, food safety, or water quality testing laboratories that possess methods, equipment, and personnel that can enhance the environmental testing capacity of their HAI or AR pathogen laboratories.

Diverse environmental samples may be analyzed to support outbreak investigations. Samples from inanimate objects in the outbreak setting, such as hospital furniture, water fixtures and equipment, and water and cooling systems, may be collected using swabs. Air samples may be of interest during invasive fungal infections. Outbreak responders may want to consult with laboratorians regarding the ecology of the targeted organism to help develop epidemiologic hypotheses and guide sample collection. Additionally, identification of a laboratory's capacity not only to sample but also to process sampling devices is crucial to developing an environmental sampling strategy.

The selection of collection devices for environmental sampling depends on many factors, such as the size, porosity, hydrophobicity, and ease of downstream processing of targeted fomites and sampling devices. Swabs come in a variety of materials, such as foam, cotton, and rayon, and are ideal for sampling small surfaces and

crevices. For larger surfaces, use of a paddle, sponge, or wipe device increases the likelihood of recovering microbes. Premoistening the selected sampling device with a sterile buffer that also neutralizes any residual disinfectants will also improve the chances of recovering the outbreak organism (Table 6.3). It is ideal for environmental samples to be transported under refrigeration and processed within 24 hours after collection. Establishing and maintaining the chain of custody (COC) related to samples is especially important for outbreak investigations that may implicate medical products or devices.

Table 6.3 | Tips for Collecting Environmental Samples[11]

SAMPLE TYPE	SAMPLING DEVICE AND MECHANISM
Small surface	• Use premoistened swab.
Large surface	• Use premoistened paddle, sponge, or wipe.
Bulk water and ice	• Collect one liter.
Drinking water	• Collect one liter. • Add sodium thiosulfate to neutralize disinfectants.
Fluid from the medical device line	• Run device pumps before collection to suspend nonmotile organisms.
Medical device	• Consult with a biomedical engineer for the best collection strategy that does not adulterate the device. • Neutralize cleansers and disinfectants that may be present.

6.2.6 Healthcare Worker Testing

Healthcare workers occasionally are screened during outbreaks, particularly in those outbreaks involving methicillin-resistant *Staphylococcus aureus* or *Streptococcus pyogenes*. Screening methods are well established for these two organisms, but for many others (such as multidrug-resistant Gram-negative organisms), methods are still under development and will continue to evolve as more complex resistance phenotypes emerge.

Results may be difficult to interpret, because recovery of outbreak pathogens from screening cultures obtained from healthcare workers does not establish the direction of transmission or definitively implicate workers as the source of the outbreak. Also, culturing samples from healthcare workers is a fraught procedure and may be perceived as hostile if mandated. Healthcare worker testing may fall under human subjects testing, which requires institutional review board (IRB) approval and has potential legal ramifications. Healthcare workers should therefore be screened only after consultation with an epidemiologist experienced in outbreak investigation; and screening should ideally be made in groups of workers with similar roles to focus interventions on practices rather than individuals. Additionally, healthcare providers should be engaged and consulted, as appropriate, in addressing the health concerns or treatment needs of individual healthcare workers who are being tested.

6.3 Safety, Quality Control, and Validation

Quality testing in a safe environment is a primary goal in any laboratory, but the processing of AR, novel, and emerging pathogens contributes complexities that can increase turnaround time for reporting. The impacts of self-infection or laboratory contamination with these organisms can compromise health or the integrity of the testing space, respectively; laboratorians, therefore, may take extra precautions such as wearing additional personal protective equipment (PPE) and working in a laboratory with a heightened safety infrastructure. Donning, doffing, and decontaminating PPE and working in an enhanced safety environment all increase the amount of time required to safely process a specimen.

Similarly, working with AR, novel, and emerging pathogens requires the use of quality controls that may not be readily available to non-reference laboratories; additional time may be needed to acquire the proper control materials. Finally, laboratory testing of these organisms is rapidly evolving. Several tests have not received the required FDA approval or have been developed at a laboratory (laboratory-developed test [LDT]), which would require validation by the user prior to use, often requiring considerable time.

6.4 Laboratory Data Management

Laboratory data can play a significant role in detecting an outbreak that involves healthcare-associated drug-resistant pathogens. Laboratory information systems (LISs) are software systems used by most laboratories to process, manage, and store data. The electronic centralization of data provides a mechanism for rapid analyses of large datasets and identification of trends. Some LISs can be configured to send alerts to remind laboratory personnel to save an isolate when it meets predefined criteria and to generate reports that identify patients with specific test results. These reports can be used to help identify cases and isolates that should be saved for additional analysis such as sequencing. Some national networks and resources managed by CDC that may be of assistance in this area are found below:

- National Healthcare Safety Network (NHSN): One function of this system is to tracks HAIs.
- Emerging Infections Programs (EIP): This national resource provides surveillance, prevention, and control of emerging infectious diseases.
- Healthcare-associated infections – community interface (HAIC): This network of state health departments and academic medical center partners provides information on emerging HAI threats, advanced tracking methods, and AR pathogens in the US.

Other suggested best practices for using laboratory data include the following:

- Communicate routinely with state epidemiology/ healthcare-associated infection programs, hospital infectious diseases and infection control departments, microbiology laboratory directors, and other key partners.
- Compile and report significant and unusual findings of drug-resistant organisms to individual healthcare facilities' infection control departments on a regular (weekly/monthly) basis.
- Generate an annual statewide antibiogram that can be shared with healthcare facilities.
- Share characterization data (i.e., those provided by NGS) of highly drug-resistant or rare isolates.

6.4.1 Ensuring Chain of Custody

A chain-of-custody (COC) document should accompany all sample handling from receipt through disposition ("cradle to grave") with the goal of preventing any opportunity for tampering. In this section, we do not provide comprehensive guidance regarding chain of custody. Rather, our intention is to provide an awareness of the utility of a COC document in the context of AR pathogens and HAIs as well as general information for consideration.

A COC document may not be common practice for laboratorians primarily involved in clinical laboratory testing of AR pathogens and/or HAIs; however, there are situations in which it may be prudent to have one or one may be requested by a submitter. For example, if a pathogen with a novel resistance profile—one that has the potential to severely threaten the public's health—is identified, a laboratory may elect to implement an internal COC document to prevent theft and misuse. HAI investigations in which law enforcement is involved due to negligence, intentional harm, or otherwise, may prompt the submission of a sample already covered by COC documentation.

While each laboratory's resources and needs are unique, there are critical elements of COC documentation and procedures that are standard, including the following: 1) the submitter's contact information; 2) description of the evidence; 3) signatures for transfer of custody; and 4) documentation of final disposition of the sample. To strengthen recordkeeping in support of the chain of custody, laboratories may photograph the evidence, document and track aliquot transfers, document disposition, document communications, and compile all resulting records in a single "case file" for ease of retrieval. However, a laboratory decides to proceed, it is the quality, not the quantity, of documentation that is paramount in a COC document, and this is critical to the legal defensibility of the data generated.

6.5 Epidemiology-Laboratory Communication

Communication between laboratorians and epidemiologists during all stages of an outbreak is crucial for comprehensive and suitable public health action. Communication should begin as soon as possible to ensure proper specimen collection and accurate laboratory test results. Before specimen collection, laboratorians can advise on relevant factors

for consideration, including the sample type to be collected, specific storage medium and conditions, time constraints to ensure sample viability, and testing turnaround time. Clinical samples from residents or patients and environmental samples from the facility and equipment may be suitable; appropriate collection and storage guidance is vital because incorrect temperatures and inappropriate conditions may negatively influence laboratory results. Some outbreak investigations may require testing in specialized laboratories. The PHL will be able to facilitate specimen collection, testing, and reporting of results. The PHL can serve as the single point of contact for all partners throughout the investigation. Coordination and communication are critical, especially when multiple facilities and laboratories are involved. Thus, effective communication with testing laboratories at the outset is necessary to understand the specific needs of an outbreak investigation and to prepare for all potential challenges. Optimally, channels of communication should be established and relationships fostered prior to an incident to facilitate an expedited response.

The laboratory's ability to respond to an outbreak can vary depending on available reagents and supplies, and even on personnel. Once the scope of an outbreak has been determined, additional laboratory staff may need to be trained. Existing protocols may require modifications, including additional validation or verification. This highlights the importance of early communication between the laboratory and epidemiology. Laboratorians and epidemiologists should coordinate specimen collection and delivery to the lab as well as the expected timeline for the availability of results. For example, specimens collected on a Thursday and received by the lab on a Friday may require additional weekend staff for processing and testing. It may be better to collect specimens on a Wednesday, so that the results can be reported before the weekend. Thus, communication between epidemiology and the laboratory should occur through an open channel to ensure priorities are met without compromising testing quality and results.

6.5.1 Other Testing

There are occasions when it is necessary to investigate an outbreak or suspected outbreak of an organism other than those mentioned in this chapter. In those cases, it is again crucial to maintain the proper chain of custody of all samples and specimens, and to ensure the proper quality control of all testing. Communication is vital to ensuring a timely and accurate response to every outbreak. Other outbreak investigations may involve toxin testing for endotoxin using LAL and gel clot, *Staphylococcus* exfoliative toxin, or *Clostridioides difficile toxin*; sterility testing using USP 71 or USP 61 for non-sterile products; or histopathological analysis of samples.

6.6 Quality Control and Assurance

As with all laboratory testing, in addition to appropriate regulatory certifications, quality control and assurance are vital to ensuring actionable and timely results. Commercial reagents and FDA-approved kit-based tests need to be quality checked, as described in their package inserts. Before beginning any new method, proper validation or verification of the method must be completed. Methods can vary by jurisdiction, but general principles apply. There must be a written plan that includes the number of isolates or specimens evaluated, as well as the acceptance criteria for sensitivity and specificity, accuracy and precision, and inter- and intra-run variability. The plan and final report must be approved and signed by the laboratory director. All tests must include appropriate positive and negative controls, as described in the test package insert, following relevant CLSI guidance and in accordance with Clinical Laboratory Improvement Amendments (CLIA) standards. All tests must be performed in the manner described in standard operating procedures. Results must be checked for accuracy prior to reporting. When performing PCR and sequencing involving amplified material, best practice is to conduct "wipe tests" of the environment to rule out contamination. Unusual results or drug-resistance patterns as well as results that are not reproducible should be discussed with the laboratory director and quality manager before action is taken.

CORHA Keys to Success

Laboratory as a Key Team Member

- Perform clinical testing:
  - Support and/or confirm diagnosis; and
  - Detect outbreak.
- Perform environmental testing:
  - Determine the outbreak source.
- Perform organism identification.
- Perform AST.
- Identify novel AR patterns.
- Identify clusters of illness and potential outbreaks.
- Perform advanced testing, as able and appropriate, to determine the relatedness of clinical cases.
- Determine the mode of pathogen transmission.
- Collaborate with other laboratories (PHLs and reference laboratories), epidemiologists, and hospital infection prevention (IP) staff to ensure adequate capability and capacity to respond to HAIs and established as well as emerging AR pathogens.
- Provide sample collection and shipping materials, including any required requisition forms and guidance for specimen transport.
- Transport specimens to reference, environmental, or other specialized laboratory testing facilities, as necessary.
- Communicate reportable HAIs and AR pathogens to appropriate authorities, including local epidemiology centers.
- Participate in AR surveillance (local, state, and federal) to support rapid identification of novel AR pathogens and early outbreak identification in order to prevent additional illness and spread of infection.
- Provide interpretation of laboratory test results and technical consultation to epidemiologists, public health members, healthcare workers, hospital IP staff, and others
  - To guide/focus investigations;
  - To assist with the development of case definitions; and
  - To identify the appropriate number and type of specimens for collection.
- Host visiting epidemiologists and/or hospital IP staff during rounds.
- Store samples, as able and requested, to support additional testing requests.
- Maintain a chain of custody of samples, as necessary.
- Employ electronic laboratory reporting for rapid communication of quality data.
- Use LIS to mine data and assist epidemiologists and hospital IP staff in the identification of trends.
- Communicate routinely with other outbreak team members to understand the needs and roles of all participants.

CORHA Keys to Success

Appropriate and Rapid Testing

1. Communication between partners is crucial and must begin early.
2. Communications concerning the expected number of specimens, collection date, transport, and expected turnaround time should be clear.
3. Results and reports should be shared in real time.
4. Sequencing can play a pivotal role in the detection of novel resistance mechanisms and determination of relatedness between strains.

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