DIAGNOSTIC MICROBIOLOGY UPDATES

General Principles of Specimen Collection and Transport

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In this issue of Clinical Infectious Diseases, *we present the first article in a series entitled "Diagnostic Microbiology Updates." Although clinical microbiology is included in the curricula of virtually all infectious disease fellowships, the degree of emphasis on this subject varies considerably. Infectious disease physicians—even those who have direct responsibities or consulting responsibilities for the microbiology laboratories of the institutions in which they practice—may be hard pressed to keep up with the rapidly changing content of the primary literature in clinical microbiology. The purpose of this series, therefore, is at least in part to fill this void and to provide concise updates for clinicians. The first article, written by Dr. Michael L. Wilson, reviews current concepts in specimen collection and transport. A key issue for all clinicians (which is not always sufficiently emphasized) is the quality of the specimen submitted to the laboratory. It is an axiom that if specimens of poor quality are submitted, the results generated by the laboratory will have little or no clinical utility. Dr. Wilson's article describes some of the methods available to assure that only specimens of good quality, i.e., those most likely to be useful clinically, are processed in the microbiology laboratory. Future articles will address specific types of specimens, groups of pathogens, and diagnostic techniques, including molecular methods. We hope this series will be informative and valuable to the readers of* Clinical Infectious Diseases, *and we look forward to your comments.*

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Specimens submitted for microbiological testing require proper handling from the time of collection through all stages of transport, storage, and processing. Issues common to all clinical specimens submitted for microbiological testing include not only proper identification but also collection techniques that maximize recovery of microbial pathogens and minimize contamination. For specimens such as sputum and urine, the relative proportions of microorganisms present in vivo must be preserved, or culture results may be misleading. If specimens are handled properly, culture results are easier to interpret, patient care is improved, and costs are potentially decreased. Although most guidelines for specimen handling remain unchanged, a recent emphasis has been placed on modifying traditional practices to decrease or eliminate unnecessary work, increase laboratory efficiency, and make microbiological testing more cost effective.

Proper handling of specimens is crucial for obtaining microbiological test results that are both timely and clinically relevant. Proper handling of specimens is also one of the most important factors—along with appropriate use of tests—in maximizing the cost-effectiveness and clinical relevance of microbiological testing. The purpose of this article is to review recent changes in specimen handling, particularly specimen collection, that can be used to modify traditional practices in clinical microbiology [1].

General information on the collection, transport, and storage of specimens from different body sites for microbiological testing is presented in table 1. More-specific information is presented in the references from which this table was derived [2–6].

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General Principles

Collection. Several axiomatic principles guide the collection of specimens for microbiological testing (table 2). The most self-evident of these principles is that specimens must be collected with use of strict aseptic technique from anatomic sites most likely to yield pathogenic microorganisms. Even so, it is surprising how often microbiology laboratories receive specimens collected from sites that are inappropriate for testing. Common examples include sinus tract specimens from patients with suspected osteomyelitis, surface material from decubitus ulcers or diabetic foot ulcers, nasal swab specimens from patients with suspected sinusitis, and 24-hour collections of urine. Laboratory personnel and clinical staff should define carefully which types of specimens are appropriate for testing.

Specimens should be collected in such a way that contamination by indigenous flora is minimized. This is of paramount importance for cultures of blood, bone, and other tissues or fluids in which infection is often caused by indigenous flora and for specimens collected from sites of putative infection that are contiguous to, or immediately adjacent to, cutaneous or mucosal surfaces.

Sufficient material must be submitted for cultures and other tests; laboratory staff are often asked to perform routine cultures, anaerobic cultures, or fungal and mycobacterial cultures on specimens consisting only of swabs or a few drops of fluid. Volume, while important for all specimens, is crucial for blood and for mycobacterial and fungal cultures of CSF and urine.

Whenever possible, tissue or fluid should be submitted for culture; with a few obvious exceptions (e.g., throat cultures and urethral cultures), swab specimens are unacceptable. While swabs have the advantage of being convenient and easy to use, they limit the volume of specimen that can be collected, they can compromise a direct gram stain, they become contaminated easily, and they can adversely affect recovery of certain microorganisms [5]. Although occasions do occur when collection of tissue or fluid is not possible and swabs must therefore be used, this should be an infrequent event in routine patient care.

Persons collecting specimens should provide complete information on specimen requisition forms or in computerized order-entry systems. Important information includes (1) the specific site(s) from which specimens were collected; (2) whether the patient was receiving antimicrobial therapy prior to specimen collection or at the time specimens were collected; (3) specific pathogens that are being sought; (4) the methods by which specimens were collected; and (5) whether the patient may be infected with pathogens known to be hazardous to laboratory personnel (e.g., *Brucella* or *Mycobacterium tuberculosis*). Such information is necessary to ensure that specimens are processed promptly, that appropriate cultures are performed, that test processing is appropriate for the method of specimen collection (e.g., urine obtained via suprapubic aspiration vs. the clean-catch method), and that laboratory personnel are not inadvertently and unknowingly exposed to highly pathogenic microorganisms.

Test requisitions should specify whether separate specimens have been submitted for culture and for histopathologic or cytopathologic examination or whether the laboratory staff needs to divide the specimen. In general, it is preferable for specimens to be divided in the laboratory, since this provides an opportunity for the staff and the pathologists to examine specimens to determine which portions should be cultured and which should be processed for pathological examination; this is particularly important in terms of infectious diseases for which histopathologic examination can yield rapid preliminary or definitive identification of etiologic agents. Even if microbial identification is not possible, classification of characteristic tissue reactions yields important clues to diagnosis, may be used to modify microbiological testing, and can be used to guide or modify empirical antimicrobial therapy. Finally, it is important that the pathologist grossly examine tissue specimens to determine if other disease processes are present because the gross morphological features associated with some infectious diseases overlap those associated with other types of disease.

Transport. In general, specimens collected for microbiological testing can be transported in sterile specimen containers or, in the case of fluid specimens, in the syringe in which the specimen was collected. This is especially true for specimens collected from hospitalized patients, since transportation to the laboratory is generally faster and more reliable than it is for specimens collected from physicians' offices, clinics, or off-site facilities. If transport of a specimen will be delayed, use of a transport device may be necessary to optimize testing. Many commercial products are available for transporting tissue and/or fluid specimens.

Specimens for culture should be transported to the laboratory as promptly as possible for processing. Health care systems should have an infrastructure that eliminates systematic delays in either transport or processing; unavoidable delays must be minimized. Specimen containers must be transported in such a way as to minimize damage that could result in contamination of the specimen or exposure of personnel to blood or other body fluids. Most specimens can be transported at room temperature. Some specimens must be transported on ice (table 1). For health care systems with off-site facilities, transportation procedures should be developed to minimize delays, prevent damage to specimens during transport, and prevent loss of specimens.

Storage. Most specimens requiring prolonged storage before processing should be refrigerated. Refrigeration maintains the viability of pathogens and preserves them in their relative proportions. The latter factor is crucial when semiquantitative cultures or quantitative cultures (e.g., cultures of sputum or urine) are necessary for interpretation of results. Refrigeration also minimizes the growth of contaminants. Specimens that should not be refrigerated include blood, which should be kept at room temperature or in an incubator at 35°C; CSF, which, with the exception of that collected for viral cultures, should be transported at room temperature; and specimens submitted for culture of *Neisseria* species, which should be transported

Table 1. Guidelines for collection, transport, and storage of specimens for microbiological testing.

Specimen or site	Container or method	Volume (mL)	No. of specimens	Transport (temperature, time)	Storage (temperature)	Comments
Blood						
Type of test						
Routine	Blood culture vials (aerobic and anaerobic) or lysis-centrifugation tube	20–30 for adults, 1–5 for children	2–3	25°C	25°C or 35°C	Avoid delays in processing lysis-centrifugation tubes
Fungal	Aerobic blood culture vials, BACTEC HBV-FM* vials, or lysis-centrifugation tube	20–30 for adults	2–3	25°C	25°C or 35°C	...
Mycobacterial	BACTEC 13A or lysis-centrifugation tube	10–20	1	25°C	25°C or 35°C	Submit one specimen initially; repeat if negative but mycobacteremia is clinically suspected
Bone	Sterile vial	NA	NA	25°C	4°C	Only infected bone should be cultured; avoid contamination from sinus tracts or skin
Catheters						
Type of catheter						
Urinary	NA	NA	NA	NA	NA	Specimen is inappropriate for culture
Vascular	Catheter tip in sterile vial	NA	NA	25°C	4°C	For detection of line sepsis, draw peripheral blood for cultures
CNS						
Abscess fluid	Anaerobic vial	1–5	NA	25°C	4°C	...
CSF	Sterile vial	1–5	NA	25°C (4°C for viral cultures)	4°C for ≤24–48 h (-70°C for ≥48 h for viral cultures)	Cytomegalovirus loses infectivity if stored at −20°C and with freeze-thaw cycles
Shunt/catheter fluid	Sterile vial	1–5	NA	25°C	4°C	...
Tissue	Anaerobic vial	NA	NA	25°C	4°C	...
Eye	Sterile vial	NA	NA	25°C	4°C	For bacterial cultures, handle in same way as other tissues
Fluid						
Abdominal	Anaerobic vial	1–10	NA	25°C	4°C	...
Pericardial	Anaerobic vial	1–10	NA	25°C	4°C	...
Pleural	Anaerobic vial	1–10	NA	25°C	4°C	...
Synovial	Anaerobic vial	1–10	NA	25°C	4°C	...
Other	Anaerobic vial	As appropriate	NA	25°C	4°C	...
Genitourinary tract (STDs)						
Organism to be cultured						
Candida albicans	Swab	NA	NA	NA	NA	...
Chlamydia trachomatis	Swab (transfer contents to 2-sucrose phosphate solution)	NA	NA	4°C	4°C	Avoid use of cotton swabs
Haemophilus ducreyi	Swab	NA	NA	NA	NA	...
Herpes simplex virus	Swab	NA	NA	NA	4°C	Avoid use of calcium alginate swabs and swabs on wooden applicator sticks
Mycoplasma/ Ureaplasma	Swab (transfer contents to 2-sucrose phosphate)	NA	NA	NA	NA	Avoid use of calcium alginate swabs and swabs on wooden applicator sticks
Neisseria gonorrhoeae	Insulate medium immediately	NA	NA	25°C	None; incubate immediately	Various transport systems are available; avoid calcium alginate swabs
Treponema pallidum	Scraping or aspirate of lesions on slide	NA	NA	Transport immediately	NA	Perform darkfield microscopic examination immediately
Trichomonas vaginalis	Swab for culture, smears for rapid tests	NA	NA	NA	NA	...

Table 1. (*Continued*)

Specimen or site	Container or method	Volume (mL)	No. of specimens	Transport (temperature, time)	Storage (temperature)	Comments
Genitourinary tract (diseases other than STDs)						
Specimen or site of specimen collection						
Amniotic fluid	Anaerobic vial	1–10	NA	25°C	4°C	. . .
Cervix	Swab	NA	NA	NA	NA	Specimen is unacceptable for anaerobic culture
Endometrium	Anaerobic vial	1–5	NA	25°C	4°C	. . .
Pelvic fluid (culdocentesis fluid/abscess)	Anaerobic vial	1–5	NA	25°C	4°C	. . .
Prostate	Obtain secretions via prostatic massage (use sterile vial)	1–5	NA	25°C	4°C	Specimen is unacceptable for anaerobic culture
Vagina	Swab	NA	NA	25°C	4°C	Specimen is unacceptable for anaerobic culture
Hair	Sterile vial or Petri dish	NA	NA	NA	NA	. . .
Oral cavity	Anaerobic vial	NA	NA	25°C	4°C	Submit tissue or fluid collected from site of infection; collect specimen in such a way as to eliminate or minimize contamination with oral flora
Respiratory tract						
Specimen or site of specimen collection						
Bronchoscopy fluid	Sterile vial	NA	NA	25°C		Specimen is unacceptable for anaerobic culture unless collected with protected catheter
Expectorated sputum	Sterile vial	NA	NA	25°C	4°C	Specimen is unacceptable for anaerobic culture; screen for contamination with saliva
Nasopharynx	Swab	NA	NA	25°C (plate immediately)	4°C	Specimen is unacceptable for anaerobic culture
Sinuses	Aspirate transferred to anaerobic vial	NA	NA	25°C	4°C	. . .
Throat	Swab	NA	NA	25°C (plate immediately)	4°C	Specimen is unacceptable for anaerobic culture
Skin and soft tissues						
Site of collection or organism to be cultured						
Deep wound/abscess	Anaerobic vial, syringe	NA	NA	25°C	4°C	. . .
Dermatophytes	Sterile Petri dish	NA	NA	25°C	4°C	. . .
Superficial wound	Anaerobic vial, syringe	NA	NA	25°C	4°C	Do not submit swabs of specimens from the surface of decubitus ulcers, diabetic foot ulcers, margins of nonviable amputations, or other wounds
Stool						
Type of test						
Culture	Sterile screw-capped jar or container	NA	1–3	Immediately	4°C for fresh specimen	Do not submit specimens from patients who develop diarrhea after 3–4 days of hospitalization; submit specimen for detection of *Clostridium difficile*
Ova and parasite examination	Sterile screw-capped jar or container	NA	1 (initially)	Immediately for fresh specimen; commercial system for preserved specimens	4°C for fresh specimen	

Table 1. (*Continued*)

Specimen or site	Container or method	Volume (mL)	No. of specimens	Transport (temperature, time)	Storage (temperature)	Comments
Urine						For all specimens, submit >20 mL of urine for mycobacterial or fungal cultures
Specimen or site						
Clean-catch	Sterile vial	1–20	NA	Immediately at 4°C or 25°C	4°C	Specimen is unacceptable for anaerobic culture
Indwelling catheter	Not acceptable for culture	NA	NA	NA	NA	. . .
Straight catheter	Sterile vial	1–20	NA	Immediately at 4°C or 25°C	4°C	Specimen is unacceptable for anaerobic culture
Suprapubic aspirate	Anaerobic vial	1–20	NA	Immediately at 4°C or 25°C	4°C	Specimen is acceptable for anaerobic culture

NOTE. Data are from [2–6]. NA = not applicable; STDs = sexually transmitted diseases.

* BACTEC high-blood-volume fungal medium (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD).

in an atmosphere with sufficient CO_2 and humidity and in a manner that prevents wide temperature fluctuations.

Written guidelines. Clinical microbiology laboratories should distribute written guidelines for proper specimen collection, transport, and storage. These guidelines should be complete, explicit, and up-to-date. Copies should be available to all personnel who handle specimens. Identical guidelines should be included in the laboratory procedure manual, with copies available for laboratory staff who answer telephone queries. In laboratories with sufficient computer resources, particularly those with a computerized order-entry system, guidelines can appear as a prompt when a test is ordered. In teaching institutions, new house staff should be given written instructions on handling specimens as part of their orientation.

Rejection criteria. Accredited laboratories must specify rejection criteria for specimens that are collected, transported, or stored under improper conditions prior to processing. Examples of specimens that are unacceptable for processing are listed in table 3. Clinical laboratories should be nearly inflexible regarding this issue; there is no benefit—and there is the potential for harm—to patients when specimens that have been improperly collected or improperly transported are processed and test results are reported. Correct labeling is of particular importance for ensuring that patient misidentification does not occur and that appropriate testing is performed. In all instances, the physician who ordered the test, as well as the person who collected the specimen, should be notified when a specimen is rejected.

Table 2. Principles of specimen collection for microbiological testing.

- To minimize contamination, use strict aseptic technique when collecting specimens
- Collect specimens from anatomic sites most likely to yield pathogens and least likely to yield contaminants
- Tissue or fluid submitted for culture is always superior to material on swabs
- Submit adequate volumes of specimens
- Provide complete information on specimen requisition forms or during entry of electronic orders
- Notify microbiology laboratory and surgical pathology laboratory when there is a need for both culture and histopathologic examination

Blood

Accurate and timely detection of bacteremia and fungemia remains one of the most important functions of clinical microbiology laboratories. For more than any other type of specimen, pre-laboratory (pre-analytic) variables affect microbial recovery, contamination rates, and the ability of physicians to interpret test results. Although a subsequent article in this series will be devoted entirely to the subject of blood cultures, it is important to emphasize that proper collection and transport of blood for culture are crucial.

Collection. The clinical interpretation of blood culture results, as well as the cost-effectiveness of blood cultures, depends on many variables; of these variables, the most important is the proportion of blood cultures that are contaminated by skin flora [7, 8]. Since only 8%–9% of blood cultures yield microorganisms that are ultimately judged to be the cause of an episode of bacteremia or fungemia, it is imperative that

Table 3. Specimens that are unacceptable for microbiological testing.

- Unlabeled or improperly labeled specimens
- Specimens received in leaking, cracked, or broken containers
- Specimens with obvious (visually apparent) contamination
- Unpreserved specimens received >12 hours after being collected
- Specimens not appropriate for a particular test

Table 4. Comparisons of disinfectants used for blood cultures.

Reference	Disinfectant	No. of cultures	No. (%) of pathogens	No. (%) of contaminants
[13]	Isopropyl alcohol (applied twice)	1,609	159 (9.9)	18 (1.1)
	Tincture iodine/ isopropyl alcohol	179	15 (8.4)	2 (1.1)
[15]	Isopropyl alcohol/ povidone iodine	181	12 (3.3)*	8 (4.4)
	Isopropyl alcohol	181		6 (3.3)
[16]	Povidone iodine	4,139	626 (7.4)*	259 (6.3)
	Tincture iodine	4,328		162 (3.7)†
[14]	Isopropyl alcohol/ povidone iodine	763	104 (13.6)	35 (4.6)
	PREP‡	783	114 (14.6)	17 (2.2)§

* Total no. (%) of pathogens recovered during both phases of each study.
† $P < .00001$.
‡ Consists of 2% iodine tincture and isopropyl alcohol swabs (Mediflex Hospital Systems, Overland Park, KS).
§ $P < .01$.

contamination rates be minimized. Even with good collection technique, 1%–3% of blood cultures are found to be contaminated. In some clinical settings (e.g., teaching hospitals and emergency departments), blood culture contamination rates are much higher, compromising the ability of physicians to distinguish between contaminants and clinically important isolates. Blood culture contamination rates can be minimized by strict adherence to aseptic collection technique and, whenever possible, collection of peripheral blood via venipuncture rather than via indwelling vascular catheters [9].

Although the results of a recent meta-analysis [9a] suggest that changing needles prior to inoculating blood culture bottles results in a statistically significant decrease in contamination rates, the results of several other studies do not support this conclusion [10–12]. Moreover, switching needles increases the likelihood of a needle-stick injury [12]. In the absence of an unequivocal benefit that outweighs the potential risk of needle-stick injury, this practice should be proscribed.

At most institutions, an iodophor or tincture of iodine, alone or in combination with isopropyl alcohol, is used for disinfecting skin. Despite the clinical importance of the choice of disinfectant, few controlled comparisons of different disinfectants have been performed (table 4) [13–16]. Even though two studies [14, 16] have shown statistically significant differences in the effectiveness of disinfectants, taken together the results of all the evaluations suggest that the specific disinfectant used may be less important with regard to contamination of blood cultures than is good disinfection technique.

Number of cultures. If sufficient blood (20–30 mL) is drawn from each venipuncture site, virtually all septic episodes can be detected with two-to-three blood cultures [17, 18]. This is true both for septic episodes characterized by intermittent bacteremia and those characterized by continuous bacteremia. For patients with suspected infective endocarditis that is likely to be caused by indigenous bacteria, the performance of three or four cultures may be necessary before the physician can be certain of the clinical importance of any isolates that are recovered [7]. As cultures of blood drawn from patients with continuous bacteremia almost always yield microorganisms, it is rarely necessary to perform more than three or four cultures. The once-common practice of ordering "blood cultures times six" in cases of suspected infective endocarditis should be abandoned, as this practice contributes nothing to patient care, and it is costly and wasteful of resources and needlessly contributes to nosocomial anemia. On the other hand, drawing only a single sample of blood for routine bacterial and fungal cultures is inappropriate [19]; single blood cultures will not reliably result in detection of all septic episodes, and such a practice compromises the ability of physicians to interpret blood culture results. Receipt of single blood samples in the laboratory should result in notification of the physician that additional samples are needed [19].

In contrast, when blood for cultures is drawn for the purpose of detecting disseminated *Mycobacterium avium* complex (MAC) infection, single samples should be drawn initially [20]. Although drawing two samples has been shown to increase yield [20, 21], a second blood sample is unnecessary since the first culture yields the result (positive or negative) for 98% of specimens [20]. Lack of a final result for the other 2% of specimens is generally acceptable in terms of patient care, since the clinical course of MAC bacteremia is more indolent than that of bacteremia caused by other bacteria or fungi; a second blood culture is not needed to interpret mycobacterial blood culture results (i.e., mycobacteria are virtually never contaminants); and second specimens can be collected easily. Therefore, a second specimen should be drawn only if the first culture is negative and the clinical findings continue to suggest disseminated MAC infection.

Volume of blood cultured. Because of the low number of microorganisms present in the blood of adults who are bacteremic or fungemic, the most important variable in recovering bacteria or fungi from adults is the volume of blood cultured [22, 23]. For adults, the recommended volume of blood to be drawn for *each* blood culture (i.e., from each venipuncture site) is 20–30 mL. For infants and small children, the number of organisms in the blood can be, but is not always, higher [24]. Therefore, although bacteremia or fungemia can be reliably detected when small volumes of blood ($\leq$1 mL) are cultured, microbial recovery is enhanced when larger volumes of blood are cultured [25]. For infants and small children, the recommended volume is 1–5 mL. For older children, the volume of blood to be drawn per culture should be appropriate for the age and weight of the patient.

Culturing an adequate volume of blood also ensures that the proper ratio of blood to broth medium is attained within each bottle. Although this ratio is probably less important than volume per se in optimizing microbial recovery, maintaining a

blood-to-broth ratio of between 1:5 and 1:10 enhances microbial recovery [26, 27].

Timing of collection. It has long been common practice to separate collection of blood specimens from a given patient by arbitrary time intervals. It is surprising that until recently no one had studied this practice systematically. In 1994, Li et al. [28] showed that drawing blood for cultures either simultaneously or over a 24-hour period resulted in similar microbial recovery rates. Since there is no benefit in obtaining blood samples at intervals and it is more practical to draw blood for a set of cultures at the same time than it is to return to the bedside to draw additional specimens, there appears to be little reason to continue this practice. Moreover, drawing blood specimens simultaneously may help ensure that the blood is drawn before antimicrobial agents are administered. One possible exception would be the collection of blood for cultures over a 24-hour period to determine whether a patient has continuous bacteremia. Although this is not a necessary practice for most patients with infective endocarditis, it may be useful when the clinical importance of isolates is unclear.

Transport and storage. Blood culture bottles should be transported immediately to the laboratory. If this is not possible, bottles can be kept at room temperature or in an incubator at a temperature of 35°C to 37°C. These bottles should never be refrigerated. Blood collected in Isolator tubes (Wampole Laboratories, Cranbury, NJ) should be processed within 8 hours, since delays in processing may decrease the microbial yield [29].

Summary. For adults, two or three blood specimens (20–30 mL each) should be drawn simultaneously from different venipuncture sites as soon as possible following the clinical events that prompted performance of cultures. For patients with suspected infective endocarditis (particularly those who have prosthetic valves and whose normal skin flora may be the source of infection), the results of three or four blood cultures may establish the presence of continuous bacteremia and help the physician determine the clinical relevance of the isolates that are recovered. For children, two or three blood specimens (at volumes appropriate for the age and weight of the patient) should be drawn. Single blood cultures for the detection of pathogens other than mycobacteria should not be done.

Cerebrospinal Fluid

The prompt, accurate diagnosis of bacterial meningitis is among the most important tasks confronting clinical microbiology laboratories. Clinical personnel and laboratory staff should carefully coordinate the handling of specimens from the time of collection through processing.

Collection. CSF must be collected by means of strict aseptic technique, both to minimize specimen contamination and to prevent introduction of bacteria into the CNS. The risk of contamination is low when the skin is adequately disinfected prior to lumbar puncture; either an iodophor or chlorhexidine can be used for disinfection. The risk of contamination is higher when CSF is collected from catheters or shunts. Such contamination is problematic, since contaminating microorganisms are likely to be the same microorganisms (e.g., coagulase-negative staphylococci) that cause many CSF catheter and/or shunt infections.

The volume of CSF needed for culture depends on the pathogens being sought. For routine bacterial cultures, a few milliliters of CSF is adequate. In contrast, for fungal and mycobacterial cultures, microbial yield is more proportional to the volume of CSF cultured. This has led to the concept of "large-volume" cultures for which as much as 10–20 mL of CSF is processed.

In 1988, Albright et al. [30] described a procedure for storing an aliquot of CSF for future testing. This procedure, known by the acronym TRAP (transport, rapid accessioning for additional procedures), has been used successfully to provide a mechanism by which testing of specimens is delayed pending results of initial screening.

Two studies have demonstrated the usefulness of the TRAP method. In both of these studies, three strategies for decreasing inappropriate testing were evaluated: physician education, optional use of screening tests prior to the testing of CSF, and mandatory use of screening tests prior to the testing of CSF [31, 32]. In the first study, CSF specimens submitted for CSF-VDRL (Venereal Disease Research Laboratory) testing were evaluated; the initial screening tests were serological tests for syphilis [32]. In the second study, CSF specimens submitted for smears and cultures for acid-fast bacilli were evaluated; the initial screening tests were determinations of the cell counts and levels of glucose and protein in CSF [33].

The results of the first study underscore the observation of Dans et al. [33] that the use of many, if not most, CSF VDRL tests is inappropriate. In both studies, significant reductions in CSF testing occurred with mandatory use of screening tests prior to CSF testing but did not occur with optional screening. Physician education alone had no effect in the first study [31]; although this strategy was effective in the second study, the decrease in testing was apparently related to a decrease in all types of testing for the presence of mycobacteria [32].

Transport and storage. CSF specimens should be transported immediately to the laboratory. Systematic delays in transport should be identified and eliminated. Laboratories should strive to report the results of initial tests within 30 minutes of receipt of the specimen in the laboratory. From collection through processing, CSF specimens (except aliquots collected for viral cultures) should not be refrigerated until initial processing is completed. Laboratorians should consider using sequential testing to reduce the number of unnecessary CSF tests.

Respiratory Tract Specimens

Collection. Expectorated sputum continues to be the most commonly collected respiratory specimen for bacterial cultures. Expectorated sputum specimens should be screened by gram staining for contamination with saliva; results should be inter-

Table 5. Criteria for rejecting specimens of expectorated sputum.

Bartlett*

Neutrophils per field (magnification, ×10)	Grade
<10	0
10–25	+1
>25	+2
Presence of mucus	+1
Squamous epithelial cells per field (magnification, ×10)	
10–25	−1
>25	−2
	Total†

Murray and Washington‡

	Squamous epithelial cells per low-power field	Neutrophils per low-power field	Perform culture
Group 1	>25	<10	No
Group 2	>25	10–25	No
Group 3	>25	>25	No
Group 4	10–25	>25	No§
Group 5	<10	>25	Yes

* Data are from [34].

† The numbers of neutrophils and squamous epithelial cells are averaged based on examination of 20–30 separate fields (magnification, ×10). The total is then calculated; final scores of ≤0 suggest contamination with saliva and/or absence of acute inflammation.

‡ Data are from [35].

§ Some laboratories set up cultures of specimens in group 4.

preted with use of criteria such as those shown in table 5 [34, 35]. If a sputum specimen is rejected, another specimen should be collected and screened in the same manner. Specimens submitted for mycobacterial culture should not be screened with use of these criteria, as the results do not reflect the likelihood that mycobacteria will be recovered [36]. Similarly, specimens submitted for culture should not be screened on the basis of the relative numbers of neutrophils and alveolar macrophages [37]. Morris et al. [38] studied the use of gram staining in screening endotracheal aspirates; if a gram stain reveals no bacteria or reveals >10 squamous epithelial cells per low-power field, the specimen should be rejected. Other respiratory tract specimens (e.g., bronchial lavage fluid) should not be rejected on the basis of criteria used for other specimens such as sputum.

Transport and storage. Because most respiratory tract specimens are likely to contain at least a few contaminating microorganisms, specimens should be transported quickly to the laboratory to minimize overgrowth of contaminants. If transportation or processing is delayed, specimens should be refrigerated. For fungal and mycobacterial cultures, prompt processing and refrigeration help prevent overgrowth of normal flora in the specimens, which complicates the recovery of pathogens.

Stool

The laboratory diagnosis of enteric infections is challenging. Problems include the number of potential pathogens; the biologic diversity of these organisms; the emergence of new pathogens; and the fact that accurate, reliable, and practical diagnostic tests have yet to be developed for many pathogens [5]. Moreover, international travel has become so common that, in some instances, epidemiological clues as to likely etiologic agents may not be as helpful as they once were. At the least, there are many more potential causes of diarrhea in travelers than in patients who have not traveled. Consequently, microbiology laboratorians must have the expertise, experience, and resources to recover and identify a variety of potential pathogens. For many laboratories, the problem is the provision of such a service in a cost-effective manner. This problem is best solved by close collaboration between laboratorians and clinical staff, with development of test utilization strategies that are appropriate for the patient population being served.

Collection. Numerous studies have verified the observation that there is minimal value in routinely performing stool cultures or microscopic examinations of stool for ova and parasites in patients who develop diarrhea after 3 or 4 days of hospitalization [39–48]. This observation holds true for both adults and children. Although the exact cutoff time (3 days vs. 4 days of hospitalization) may vary slightly depending on the specific health care setting, it is neither beneficial to patients nor cost-effective to routinely process specimens for these tests after the 4th day of hospitalization. Stool specimens collected from patients who develop diarrhea in the hospital should be tested for the presence of *Clostridium difficile.* Manabe et al. [49] have published guidelines for using clinical data and laboratory data to guide evaluation of patients with suspected *C. difficile* disease.

Similarly, there is little value in routinely testing three or more stool specimens as part of an evaluation of acute diarrhea, as the majority of published studies indicate that most pathogens are detected in the first specimen [41, 45, 47, 48, 50] (this is true for both cultures and examinations for ova and parasites). However, some studies have not supported this observation, a finding suggesting that in some settings it may be appropriate to routinely collect two or three specimens [51, 52].

This issue is a difficult one to resolve because the failure to detect pathogens in stool specimens relates partly to intermittent shedding of some intestinal pathogens as well as to current diagnostic limitations [5]. Although the testing of multiple specimens may eventually overcome the former circumstance, it cannot overcome the latter. Rather than submitting many specimens from the same patient, clinicians should consult the

laboratory for alternative diagnostic tests for specific pathogens. For example, a significant number of cases of strongyloidiasis are missed when stool specimens are examined for the presence of *Strongyloides* with use of traditional methods (direct fecal smear, formalin-ether concentration, or culture on a filter-paper strip); this organism can be detected more reliably by coproculture with use of agar plates [53].

More widespread use of newer diagnostic products such as enzyme immunoassays for *Giardia lamblia* may also obviate the need to routinely test more than one stool specimen. In most settings—and for detection of most common enteric pathogens—awaiting results for the first specimen before collecting subsequent specimens reduces the amount of unnecessary testing.

Transport. Optimal test results are obtained when microbiological testing is performed on fresh stool specimens. Because testing fresh specimens is impractical in most clinical settings (particularly outpatient settings), most stool specimens are collected and then placed in vials containing different transport media and fixatives. A variety of such products are commercially available; each system typically includes a transport medium for culture and 10% neutral-buffered formalin and polyvinyl alcohol for ova and parasite examination. For most purposes, these transport systems work well, are convenient and easy to use, and are relatively inexpensive [54].

Storage. Stool specimens submitted for culture typically are not stored for any length of time, since most laboratories set up all appropriate cultures at the time of receipt of the specimen. Only rarely is it necessary to retrieve a specimen for additional testing; such specimens should be refrigerated. Stool specimens submitted for ova and parasite examinations are typically stored at room temperature in a fixative. Specimens stored in 10% neutral-buffered formalin remain stable for many months, even when tested with some enzyme immunoassays for *G. lamblia.* Because trophozoites can deteriorate quickly in stool, even when refrigerated, fresh stool specimens submitted for ova and parasite examination should be examined within 2 hours.

Summary. For patients with acute diarrhea, one specimen should be submitted for culture and examination for ova and parasites. If these tests are negative and symptoms persist, additional specimens should be submitted for testing. For patients who develop diarrhea after the third or fourth day of hospitalization, a stool specimen should be tested for the presence of *C. difficile.*

Specimens Collected for Viral Cultures

Many commercial products are available for the collection and transport of viral culture specimens. For most of these systems, specimens are collected on swabs that are then rinsed in a broth medium (viral transport medium). Calcium alginate swabs should not be used, since they are known to adversely affect recovery of herpes simplex virus [6]. Although most viruses survive well at ambient temperature while in common transport media, recovery of viruses from specimens containing low numbers of viruses may be decreased following prolonged holding under these conditions. Therefore, it is advisable to transport specimens on ice or to keep them refrigerated. Specimens should never be exposed to temperatures higher than room temperature. Johnson [55] has reviewed the details regarding collection and transport of specimens for recovery of specific viruses. A subsequent article in this series will include a more detailed review of diagnostic virology.

Specimens Submitted for Detection of Microorganisms by Molecular Diagnostic Techniques

A variety of molecular diagnostic assays have been developed for use in clinical microbiology laboratories. Despite the potential for improving the diagnosis of infectious diseases, several important issues need to be resolved before many of these assays can be recommended for routine use. First, it is not known whether specimens submitted for molecular diagnostic testing should be screened with use of the same methods and criteria as are used for specimens submitted for culture. Second, even though molecular diagnostic assays are analytically more sensitive (i.e., they detect smaller quantities of analyte), it is not known whether they are diagnostically more sensitive (i.e., they generate fewer false-negative test results). Third, many of these tests, particularly nucleic acid amplification assays, are not yet commercially available and thus have not been standardized. "Home-brew" assays, in particular, remain poorly standardized. Last, the clinical relevance of results obtained from many assays has yet to be defined.

Controlled clinical trials to establish performance characteristics will be possible once these assays become commercially available and are more widely used. Until that time, it would be prudent to test only those specimens that are appropriate for culture, since they have already been shown to yield more pathogens with fewer contaminants. A subsequent article in this series will include a more-detailed review of molecular diagnostic techniques.

Specimens Collected for Serological Diagnosis of Infectious Diseases

For most diseases, serological testing is not a surrogate for culture or other diagnostic tests. Physicians should order serological tests sparingly, since many serological assays have limitations that often are not appreciated. The most important of these limitations include technical issues such as cross-reactivity, turnaround time (for some assays, test results are not available in a clinically relevant time frame), and the inability to distinguish between acute disease and past exposure to infectious agents on the basis of single assays. Serological testing certainly plays an important role in the treatment of patients

with infectious diseases, but only when the tests are used appropriately.

Collection. Specimens obtained for serological analysis should be collected either in sterile evacuated tubes or in serum separator tubes. Strict aseptic technique should be used during venipuncture. Adequate volumes of blood should be drawn for anticipated tests. In many cases it is prudent to draw a small additional volume of blood that can be stored for future testing.

Most clinical laboratories perform limited serological testing, forwarding many specimens to commercial reference laboratories, state laboratories, the Centers for Disease Control and Prevention, and other public health laboratories. Because of the multiplicity of assays and testing laboratories, serological testing is best facilitated when the laboratory procedure manual contains the following data for each reference laboratory: detailed information regarding infectious agents and/or diseases for which tests are available; specific assays for each agent and/or disease; types of specimens that will be accepted for testing; minimum and optimal volumes of specimens for testing; reference ranges; limitations of assays; recommendations for specimen collection; guidelines for test interpretation; and special considerations for specimen collection and transport.

It is inadvisable to collect specimens other than serum for most serological tests. Since most commercial serological tests were not developed to test specimens other than serum, standardized controls are not available, reference ranges have not been defined, and the performance characteristics (i.e., sensitivity, specificity, and positive and negative predictive values) of the tests are unknown. In addition to these technical limitations, the clinical relevance of test results for specimens other than serum is usually unknown.

Transport and storage. To prevent loss of immunologic reactivity and growth of contaminating microorganisms, specimens should be transported promptly to the laboratory and centrifuged, and the serum should be poured off and refrigerated or frozen immediately. Serum specimens that will be used within 1 week after collection can be refrigerated. Specimens that need longer storage should be frozen at −70°C. Storage of specimens at −20°C is not recommended, since some antibodies deteriorate at an unpredictable rate at that temperature. Most assays of specimens that are properly stored at −70°C yield accurate results for many months.

Safety During Handling of Specimens Collected for Microbiological Testing

Collection. To minimize the potential exposure of personnel to infectious agents, specimens should be collected with use of standard (universal) precautions [56]. In particular, blood collection should be performed in strict accordance with guidelines to prevent needle-stick injuries [57]. Collection of specimens from patients with communicable diseases, particularly those transmitted via the respiratory route, should be done under appropriate isolation conditions.

Transport. Specimens should be transported with use of standard precautions. Additional measures should be taken to ensure that specimens are not damaged during transportation, which can result in contamination or leakage of the specimen. In particular, specimens in glass containers should be transported in such a way that the risk of breakage is minimized. This is especially important when pneumatic tube systems are used for transport, as cleanup of leaked specimens within these systems is difficult and expensive.

Microbiological specimens transported via mail or other interstate couriers is subject to federal regulations; McVicar and Suen [58] have recently reviewed these regulations. If laboratory staff are uncertain as to whether shipping a given agent is regulated or as to what constitutes appropriate packaging and labeling, they should consult with the appropriate agency before packaging the agent.

Storage and processing. Once received in the laboratory, specimens should continue to be handled with use of standard precautions. Strict adherence to such precautions decreases the likelihood of exposure to blood-borne pathogens as well as pathogens being sought in specimens and those that are clinically not suspected. Although most cultures can be plated safely on a standard laboratory bench, many microbiologists prefer setting up cultures in a biological safety cabinet. This is mandatory for specimens that may contain *M. tuberculosis.* Once pathogens are propagated in the laboratory, there is further risk of developing laboratory-acquired infection [56]; etiologic agents of particular risk include *M. tuberculosis, Brucella, Francisella tularensis, Yersinia pestis, Histoplasma capsulatum,* and *Coccidioides immitis* [56, 59]. Cultures containing (or suspected of containing) one of these agents should be processed only in Class II biological safety cabinets under Biosafety Level 3 conditions [59].

Summary

Obtaining accurate and cost-effective microbiological test results is possible only when specimens are collected, transported, and stored properly. When proper procedures are followed, cultures of specimens are less likely to be contaminated and more likely to yield pathogens. Not only does this make interpretation of test results easier, but it also reduces unnecessary work and, as documented for some specimens, reduces health care costs. Proper collection includes submitting the appropriate number of specimens. It is increasingly evident that for most specimens, submission of more than the recommended number of specimens does not improve the physician's ability to interpret test results.

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