



It's a Bug's Life—Specimen Collection, Transport, and Viability

Properly collecting and transporting specimens is critical for recovering pathogens and interpreting culture results

Frank Wegerhoff

Microbial specimens need to be properly collected and transported to ensure the recovery of pathogens for subsequent analysis. Simply stated, a “bug’s life” is of paramount importance, but without giving due consideration to its “life support” system, the chances of recovering it in a viable form are greatly diminished. Very often, pathogens cannot be recovered in viable form from clinical specimens simply because they were not properly collected. Importantly, failures at this level could lead to misleading findings in the clinical laboratory and, potentially, to disastrous consequences for patients.

Differences between “Clinical Trials Microbiology” and “Clinical Hospital Microbiology”

Are there any essential differences between specimens collected during clinical trials versus those obtained on a more routine basis from hospitalized patients? How do procedures and collection devices differ?

Both kinds of specimens are very similar, and what is important in collecting specimens in hospital settings is equally important in clinical trials. In both cases, improperly collected specimens may result in analysts recovering contaminants at the expense of, or in addition to, pathogens that are infecting patients or those participating in clinical trials. Introducing contaminants into clinical specimens can lead to unnecessary health care interventions, collection of additional specimens, administration of antibiotics, prolonged hospital stays, and unnecessary costs.

In some clinical trials, clinical specimens may be tested initially at local laboratories, and any isolates that are recovered will then be sent to a central laboratory to confirm the identity of suspected pathogens and for antibiotic susceptibility testing.

For example, in a clinical study involving skin infections, an investigator may be concerned with microbial testing only for *Staphylococcus aureus* as the primary target pathogen. If this pathogen were recovered from an initial specimen, it might be sent to a central laboratory for confirmation and additional testing. During the course of the culture process, an additional organism may be isolated, but there is no simple way of determining the origin of this additional organism. However, some investigators managing clinical trials may insist on conducting full workups of these additional organisms to identify them and determine the minimum inhibitory concentrations of antibiotics to which they may be susceptible.

One of the key differences between clinical

Summary

- Specimens for microbial testing need to be properly collected, and transported to ensure recovery of pathogens for subsequent analysis.
- One of the key differences between “clinical trials microbiology” and “clinical hospital microbiology” comes from how pathogens are defined in context.
- Because some clinical trials involve shipping specimens to distant laboratories for analysis, investigators need to use appropriate transport media to preserve microbes within those specimens.

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trials microbiology and clinical hospital microbiology comes from how pathogens are defined in context (Table 1). When planning a clinical trial that involves microbiology, the principal investigators typically distinguish between target pathogens, nontarget pathogens, and normal flora. For example, in an acute sinusitis study, the target pathogens may be limited to *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Nontarget pathogens might include gram-negative rods in general, while normal flora might likely include viridans streptococci, coagulase-negative staphylococci, *Micrococcus* species, *Corynebacterium* species, *Neisseria* species, and *Bacillus* species.

This approach differs from that taken by microbiologists in a typical hospital laboratory when testing clinical specimens from patients suspected of having some kind of infection. Clinical microbiologists conducting those tests have neither the interest nor the need to isolate nontarget pathogens and normal flora, nor would they be expected to perform antimicrobial susceptibility testing on these microorganisms.

Importance of Proper Collection of Specimens for Microbial Testing

In a hospital environment, proper specimen collection procedures are critically important. Sample collection is the first step in obtaining an accurate laboratory diagnosis of an infectious disease. Thus, it is critical that personnel who collect specimens avoid contaminating them. This also holds true for microbial specimens collected during clinical trials.

A hypothetical study involving patients with bacterial conjunctivitis provides an example of a clinical trial in which the importance of good collection practices might be emphasized. For instance, the lead investigators might stipulate that samples be collected from the cul-de-sac region of the eye. Several weeks or months into the study, the microbiology culture results may show that only normal flora are being isolated. This finding could alert investigators to the fact that specimens are being obtained from the eye's upper lid margin, where normal flora reside, rather than the cul-de-sac.

In terms of optimally recovering pathogens

Table 1. Definition of pathogens for an acute sinusitis study (example)

Definition	Examples
Target pathogens	<i>Streptococcus pneumoniae</i> , <i>Streptococcus pyogenes</i> , <i>Haemophilus influenzae</i> , <i>Moraxella catarrhalis</i> , <i>Staphylococcus aureus</i>
Nontarget pathogens	Members of the Enterobacteriaceae, other Gram-negative rods
Normal flora	Viridans streptococci, coagulase-negative staphylococci, <i>Micrococcus</i> species, <i>Neisseria</i> species, <i>Bacillus</i> species

from patient samples, directly inoculating samples onto bacteriological growth media has merit. Jules Bordet and Octave Gengou, who devised the “cough plate” for recovering *Bordetella pertussis* directly from patients, first practiced this procedure about a century ago. In their case, they held an open agar plate in front of a patient’s mouth, and the plate was sprayed with cough droplets. For other approaches, specimens may be directly streaked onto plates in settings such as the emergency room, operating room, or clinic, and then sent to the laboratory for analysis.

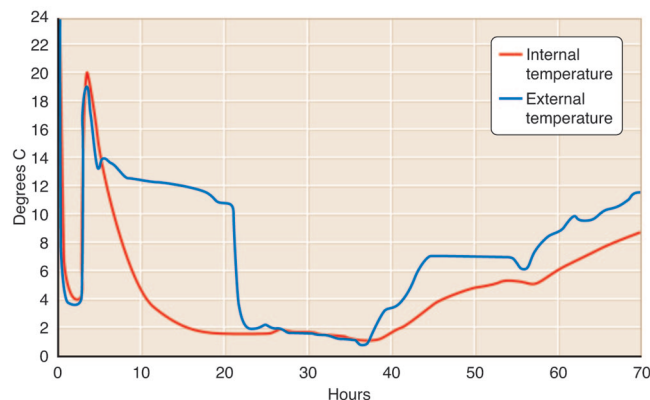
Development and Uses of Media for Transporting Microbes

Louis Pasteur (1822–1895) isolated bacterial strains and grew them in liquid media, but Robert Koch (1843–1910) was the first to develop a solid medium for growing bacteria. He experimented at first with potato slices, then gelatin, which proved unsatisfactory because it liquefies at 37°C. Soon, however, microbiologists began using an extract of Japanese seaweed called agar-agar to keep their culture media solid at 37° C. Eventually, Richard Petri (1852–1921) developed a reusable round glass dish to contain agar, and nowadays plastic disposable Petri dishes are widely used.

At the beginning of the 20th century, because there were no special devices for transporting specimens of any kind, they were submitted “as is,” with no thought given to preserving microbes. After several decades, however, experts who practiced laboratory medicine realized that



FIGURE 1



Temperature/time study. A transportation temperature between 2 and 8°C can be maintained for more than 60 hours, a critical component to shipping specimens at refrigerated temperatures from remote locations.

delays in transporting specimens containing microbes affected the recovery of those organisms—most notably, those that are considered fastidious. More generally, any failure to inoculate culture media promptly and to transport those materials properly tended to result in poor recovery of pathogens.

Robert Stuart, a public health microbiologist in Glasgow, Scotland, during the 1940s, who later moved to Edmonton, Alberta, Canada, conceived of designing a special medium for transporting clinical specimens containing microorganisms. Thus, while in Scotland, he noticed that specimens from patients suspected of having gonorrhoea failed to yield a positive culture when the specimens were stored overnight before being plated. Stuart showed that, when charcoal swabs were used for transporting such specimens, *Neisseria gonorrhoeae* survival increased. However, because those swabs were black and dusty, they proved unpopular with patients.

Stuart published a report describing his first transport medium in the *Glasgow Medical Journal* in 1946. Although designed for transporting *N. gonorrhoeae*, this medium proved excellent for transporting a wide variety of microorganisms. Stuart's transport medium is a household name in the clinical microbiology world and remains in use. C. R. Amies of the Ontario Public Health Laboratory modified Stuart's original formula, substituting an inorganic

phosphate buffer for glycerophosphate, which prevented the proliferation of coliform organisms and other gram-negative rods from throat, wound, and fecal specimens during transport.

Other transport media that have been developed include modified Stuart's, liquid Stuart's, Amies (with or without charcoal), Cary and Blair, Port-A-Cul, and A.C.T. 1. There are many variations to these transport media, but the names Stuart, Amies, Cary, and Blair are still with us.

Clinical trials involving microbiology typically are conducted on a national level, meaning institutions located in several different geographical regions, states, or provinces may be involved. Alternatively,

such trials may be conducted on a global level and involve institutions in several different countries. Whether national or global, specimens typically are shipped over long distances to a central laboratory for culture and analysis. Shipping specimens globally is more challenging than shipping them within a country because investigators must deal with more complex regulations and other factors, including import and export permits, customs, air carriers, and road carriers.

Sometimes local laboratories perform the initial culture workup before isolates are shipped to a central laboratory to confirm the provisional bacterial identification and antimicrobial susceptibility testing results. With air transport, specimens can be shipped anywhere in the world to a central laboratory within 3 days, including samples that are shipped under ambient, refrigerated, or frozen conditions.

Maintaining Viability of Microorganisms Being Transported for Analysis

What are some of the factors besides the collection procedure that affect viability and recovery of microorganisms that are transported for analysis? For one thing, the collection device is very important. When swabs are used to collect a specimen, they may be made of cotton, rayon, or dacron. Dacron and rayon swabs with plastic

shafts are not toxic to organisms, whereas cotton swabs with wooden shafts typically contain oils that inhibit the growth of some microorganisms.

Some types of transport media are better than others for particular applications. For instance, some media are designed for maintaining fastidious organisms. In clinical trials, the choice of transport medium depends in part on what target pathogens need to be recovered and also on the nature of the specimen being collected. Shipping conditions also play a significant role in determining the analytic outcome. There is not much use in shipping specimens at ambient temperatures when the organism to be tested cannot survive without being refrigerated.

Three major devices are employed to maintain viability of organisms during their shipment (Table 2). In clinical trials, patients from many different countries are often enrolled in studies, and specimens collected from these patients are shipped over vast distances. Under such circumstances, it is imperative that the viability of organisms in the specimen be maintained until they reach the central laboratory. Deciding whether to ship specimens that are frozen, refrigerated, or kept at ambient temperatures depends on a number of factors, including the type of study; countries where it is being performed; the local, national, and international shipping regulations that apply; the time of transit; and the target pathogens being sought.

Consider a project that requires shipping stool specimens from the Asia-Pacific region to a laboratory in the United States. Before testing patient specimens, investigators should demonstrate through a validation study that the enteric pathogens in such samples can be maintained for several days when shipped across long distances. In conducting validation studies, investigators customarily test American Type Culture Collection (ATCC) and clinical strains of the organisms that they are targeting. These organisms are seeded into stool specimens, which may be stored for 1 to 10 days to simulate delays in transport. Specimens may also be kept at different temperatures to determine optimal condi-

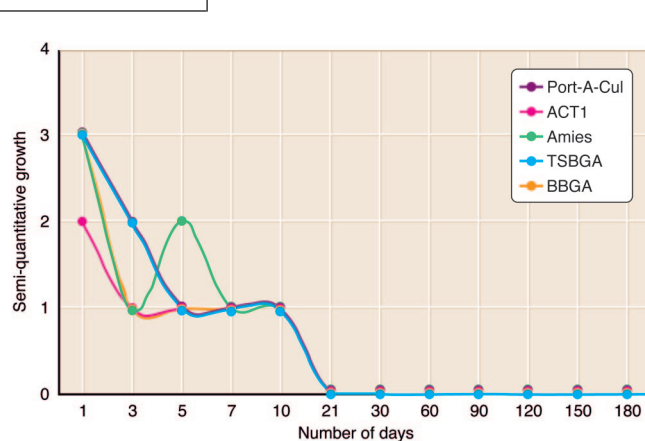
Table 2. Major devices used to maintain viability of organisms

Device	Shipping temperature	Disadvantages
Refrigeration (with gel packs)	2–8°C	Weight and cost
Freezing (with dry ice)	~ -20°C	Weight and cost
Ambient (no coolant)	20–25°C	Selective agents may have to be added to limit bacterial competition

tions for maintaining their viability. Seeded specimens are subcultured on selective bacteriological media to determine the viability of the seeded strains.

One of the dilemmas faced in transporting stool specimens is that some enteric pathogens, such as *Campylobacter* and *Yersinia*, maintain their viability better at 2–8°C than they do at 20–25°C. What is the best way to transport such specimens? The answer is to use a device with two compartments, one to refrigerate part of the sample and the other to hold another part of the sample at ambient temperature. Before such a device can be used, however, investigators should conduct another validation study to show that separate temperatures can be maintained while the specimens are in transit. Probes may be placed to track the specimen's tempera-

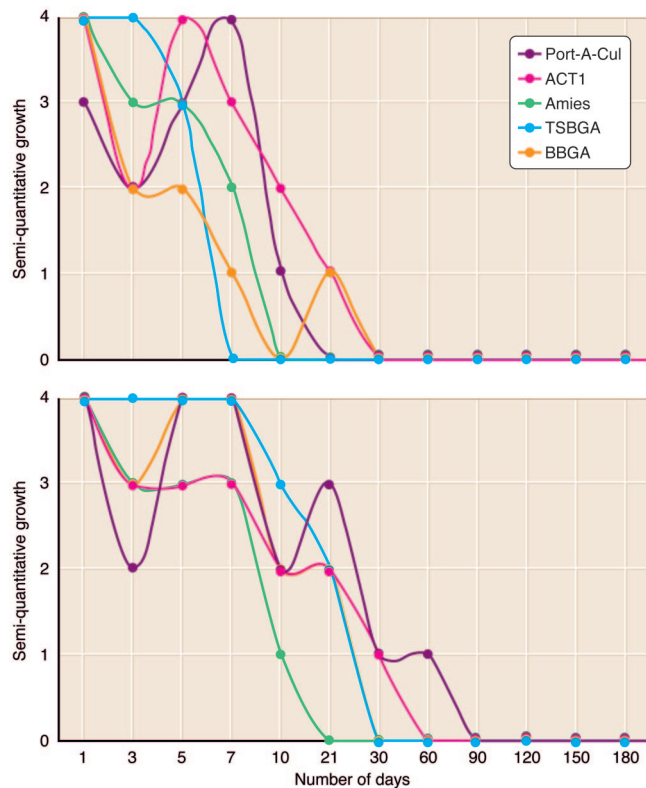
FIGURE 2



Viability of *Neisseria gonorrhoeae* under refrigeration with different types of transport media.



FIGURE 3



Viability of *Haemophilus influenzae* at room temperature and under refrigeration with different types of transport media.

ture as well as that of the external container. The probes may be used to record the internal and external temperatures at regular intervals (Fig. 1).

SUGGESTED READING

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Some clinical trials involving microbiology studies require shipping specimens from remote regions, in which case the most appropriate transport medium and shipping conditions need to be carefully selected to preserve the viability of microbes within the specimens. Validation experiments may be performed to extend a manufacturer's claims regarding the use of certain transport media. Deciding which media will maintain viability of aerobes and anaerobes under ambient and refrigerated conditions may be based on tests done with ATCC and clinical strains of organisms. Swabs to inoculate these media with the organisms under consideration may be used and then subcultured at regular intervals postinoculation, using standard bacteriological media (Fig. 2 and 3).

Concluding Comments

In recent years, clinical laboratories have shifted into a system in which many peripheral laboratories conduct only tests that can be completed the same day, while most specimens requiring culture and analysis are performed by a smaller set of “core” laboratories to which specimens are shipped. This change has led to increased reliance on the use of transport media to maintain the viability of pathogens during transit, which may take several days depending on the

distances between the peripheral and core laboratories. In clinical trials microbiology as well as in routine clinical testing, properly collecting specimens and transporting them under appropriate conditions is critically important for maintaining microbial viability.