collection and transport process coupled to a laboratory-processing algorithm suitable for the specific sample/agent. In some instances, it is better for specimens to be plated at the time of collection rather than first being transported to the laboratory (e.g., urethral swabs being cultured for *Neisseria gonorrhoeae*). In general, the more rapidly a specimen is plated onto appropriate media, the better the chance is for isolating bacterial pathogens. Deep tissue or fluid (pus) samples are more likely to give useful culture results than are superficial swab specimens. Table 150e-2 lists procedures for collection and transport of common specimens. Because there are many pathogen-specific paradigms for these procedures, it is important to seek advice from the microbiology laboratory when in doubt about a particular situation.

## ISOLATION OF BACTERIAL PATHOGENS

Isolation of pathogens from clinical material relies on the use of artificial media that support bacterial growth. Such media are composed of agar, nutrients, and sometimes substances that inhibit the growth of other bacteria. Broth is employed for growth of organisms from specimens with few bacteria, such as peritoneal dialysis fluid or CSF, or from samples in which anaerobes or other fastidious organisms may be present. Broths that allow the growth of small numbers of organisms may be subcultured onto solid medium once growth is detected. The use of liquid medium for all specimens is not worthwhile.

Two basic strategies are used to isolate pathogenic bacteria. The first is to employ enriched media that support the growth of any bacteria that may be present at a site that is normally sterile, such as blood or CSF. The second strategy is to use selective media to isolate specific bacterial species from samples that contain many bacteria under normal conditions (e.g., stool or genital tract secretions). Antimicrobial agents or other substances are incorporated into the agar medium to inhibit growth of all but the bacteria of interest. After incubation, organisms that grow on such media are characterized further to determine whether they are pathogens (Fig. 150e-1).

## BLOOD CULTURE

The detection of microbial pathogens in blood is difficult because the number of organisms present in the sample is often low and the organisms' integrity and ability to replicate may be damaged by humoral defense mechanisms or antimicrobial agents. Automated blood culture systems detect production of gas (mainly $CO_2$) by bacteria or yeasts growing in broth in a blood culture bottle. Because the bottles are monitored frequently, a positive culture often is detected more rapidly than by manual techniques, and important information, including the results of Gram's stain and preliminary susceptibility assays, can be obtained sooner. Several factors affect the yield of blood culture from bacteremic patients. Increasing the volume of blood tested increases the chance of a positive culture. For example, a sample-size increase from 10 to 20 mL of blood increases the proportion of positive cultures by ~30%, although this effect is less pronounced in patients with bacterial endocarditis. Obtaining multiple samples for culture (up to three per 24-h period) also increases the chance of detecting a bacterial pathogen. Prolonged culture and blind subculture for detection of most fastidious bacteria (e.g., HACEK organisms) are not needed with automated blood culture systems.

Automated systems also have been applied to the detection of microbial growth from specimens other than blood, such as peritoneal and other normally sterile fluids. *Mycobacterium* species can be detected in certain automated systems if appropriate liquid media are used for culture. Although automated blood culture systems are more sensitive than lysis-centrifugation methods (e.g., Isolator) for yeasts and most bacteria, lysis-centrifugation culture is recommended for filamentous fungi, *Histoplasma capsulatum*, and some fastidious bacteria (*Legionella* and *Bartonella*).

## IDENTIFICATION METHODS

Once bacteria are isolated, characteristics that are readily detectable after growth on agar media (colony size, color, hemolytic reactions, odor, microscopic appearance) may suggest a species, but definitive identification requires additional tests. Identification methods include classic biochemical phenotyping, which is still the most common approach, as well as more sophisticated methods such as mass spectrometry, gas chromatography, and nucleic acid tests (see below).

**Biochemical Phenotyping** Classic biochemical identification of bacteria entails tests for protein or carbohydrate antigens, the production of specific enzymes, the ability to metabolize specific substrates and carbon sources (such as carbohydrates), or the production of certain metabolites. Rapid versions of some of these tests are available, and many common organisms can be identified on the first day of growth. Other organisms, particularly gram-negative bacteria, require more extensive testing, either manual or automated.

Automated systems allow rapid phenotypic identification of bacterial pathogens. Most of these systems are based on biotyping techniques in which isolates are grown on multiple substrates and the reaction pattern is compared with known patterns for various bacterial species. This procedure is relatively fast. Commercially available systems include miniaturized fermentation, coding to simplify recording of results, and probability calculations for the most likely pathogens. If the biotyping approach is automated and the reading process is coupled to computer-based data analysis, rapidly growing organisms (such as Enterobacteriaceae) can be identified within hours of detection on agar plates.

Several systems use substrates for preformed bacterial enzymes for identification within 2–3 h. These systems do not rely on bacterial growth per se to determine whether a substrate has been used. They employ a heavy inoculum in which specific bacterial enzymes are present in amounts sufficient to convert substrate to product rapidly. In addition, some systems use fluorogenic substrate/end-product detection methods to increase sensitivity through signal amplification.

**Gas-Liquid Chromatography** Gas-liquid chromatography often is used to detect metabolic end products of bacterial fermentation. One common application is identification of short-chain fatty acids produced by obligate anaerobes during glucose fermentation. Because the types and relative concentrations of volatile acids differ among the various genera and species that make up this group of organisms, such information serves as a metabolic fingerprint for a particular isolate.

Gas-liquid chromatography can be coupled to a sophisticated signal-analysis software system for identification and quantitation of long-chain fatty acids (LCFAs) in the outer membranes and cell walls of bacteria and fungi. For any particular species, the types and relative concentrations of LCFAs are distinctive enough to allow differentiation even from closely related species. An organism may be identified definitively within a few hours after detection of growth on appropriate media.

**Matrix-Assisted Laser Desorption/Ionization/Time-of-Flight Mass Spectrometry (MALDI-TOF MS)** MALDI-TOF MS is a rapid and accurate method for identifying microorganisms by protein analysis. The organism is mixed with a chemical matrix, dried on a target plate, and then pulsed with a laser. The laser ionizes and vaporizes the microbial proteins, which then travel through a charged vacuum chamber to a detector. The time of flight to the detector is measured for the individual proteins, and the resulting pattern (or fingerprint) is compared with a library of known patterns for various microorganisms to identify the test organism.

The primary clinical advantage of MALDI-TOF is that it takes only minutes to identify an organism, whereas several hours are needed for conventional phenotyping. MALDI-TOF is highly accurate for identification of most bacteria and yeasts grown on solid agar or in blood culture broth. Other potential uses of MALDI-TOF include identification of bacteria and yeasts directly from clinical specimens (e.g., urine), detection of β-lactamase activity, and strain typing of bacteria; however, these applications are still under development.