Diagnostic Microbiology☆

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Glossary

Colony-forming unit Visible units counted in an agar plate count, which may be formed from a group of microorganisms rather than from one.

Cytopathic effects (CPE) Tissue deterioration caused by viruses. CPE is widely used for the identification of virus isolates in the diagnostic virology laboratory.

Darkfield microscope A microscope that has a device to scatter light from the illuminator so that the specimen appears white against a black background. It is widely used for direct examination of spirochete microorganisms.

Fluorescent microscope A microscope that uses an ultraviolet light source to illuminate specimens that will fluoresce.

Genomics A branch of biotechnology which applies molecular biology and genetics techniques to analyze the structure, function, evolution, and mapping of genomes.

Gold standard The best available approximation of the truth. It is a commonly used term, generally indicating a test method currently accepted as the most accurate test available.

Indigenous flora Microorganisms that colonize animals or humans without causing known disease.

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Metabalomics The study of measuring unique global variations of metabolites left behind by a cell, tissue, or organ from metabolic processes.
Nucleic acid probe A piece of labeled single-stranded nucleotide used to detect complementary DNA in clinical specimens or a culture and thus to specifically identify the presence of an organism identical to that used to make the probe.
Nucleotide microarray A collection of microspots, each labeled with a nucleotide probe, which can be used for screening numerous gene targets simultaneously.
Point-of-care testing (POCT) Diagnostic testing is performed at or near the site of patient care.
Probe amplification A nucleic acid amplification procedure in which many copies of the probe, which hybridizes the target nucleic acid, are made.
Proteomics The large-scale study of the structure, function, and interactions of the proteins produced by a particular cell, tissue, or organism.
Rapid plasma reagin (RPR) test Reagin is a substance made in response to a treponemal infection characterized by its ability to combine with lipids. The RPR test is a simple, rapid, nontreponemal card test for the diagnosis of syphilis.
Serodiagnosis A high or rising titer of organism-specific IgG antibodies or the presence of organism-specific IgM antibodies may suggest or confirm a diagnosis.
Shell vial culture A technique that combines cell culture and immunofluorescence assay for rapid detection of virus organisms.
Signal amplification A nucleic acid amplification procedure in which a signal or reporter molecule attached to the probe is detected, and the signal is amplified enormously.
Solid-phase immunoassay An immunoassay in which the capture antigen or antibody is attached to the inside of a plastic tube, microwell, or to the outside of a plastic bead, in a filter matrix, or some other solid support.
Target amplification A nucleic acid amplification procedure in which many copies of the nucleic acid target are made.
Transcriptomics The study of the complete set of RNA transcripts produced by a population of cells or tissue under a given environmental context.
Western blotting An immunologic technique for identification and characterization of protein antigen or antibody.

Abbreviations

CIA	Chromatographic immunoassay
CMV	Cytomegalovirus
CPE	Cytopathic effect
EIA	Enzyme immunoassay
FDA	Food and Drug Administration
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDA	Helicase-dependent amplification
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
MALDI-TOF	Matrix-assisted laser desorption/ionization time of flight
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
NASBA	Nucleic acid sequence-based amplification
PCR	Polymerase chain reaction
SDA	Strand displacement amplification
TMA	Transcription-mediated amplification
xMAP	Multianalyte profile

Defining Statement

Diagnostic microbiology determines whether suspected pathogenic microorganisms are present in specimens collected from human beings, animals, and environment. In medical practice, diagnostic microbiology is to define infectious processes and elucidate treatment options through detection, quantification, and characterization of specific pathogens. This article provides fundamental principles and techniques of diagnostic microbiology.

Development of Diagnostic Microbiology

The roots of diagnostic microbiology trace back to over three centuries when van Leeuwenhoek first observed bacteria and protozoa with his primitive microscope. However, it was not until the late 1800s that the work of Pasteur, Koch, and others ushered in the modern era of germ theory as well as the use of isolation techniques on artificial growth media. Since then, the capabilities of modern diagnostic microbiology have expanded and improved rapidly thanks to technological revolutions in microbiology, immunology, and molecular biology. A microorganism from a test sample can be detected and identified in any of five possible ways: (1) cultivation of microorganisms using artificial media or living hosts, (2) direct microscopic examination, (3) measurement of microorganism-specific immune responses, (4) detection of microorganism-specific macromolecules, especially nucleic acids, and (5) rapid antigen detection. The contrast of these techniques is summarized in Table 1, the following sections will discuss them separately.

Table 1 Methods used for microorganism diagnosis

Test	*Ease of performance*	*Turnaround time*	*Result interpretation*	*Advantages*	*Disadvantages*
Direct examination	Can be performed in routine clinical laboratory and in nurse station	1–3 h	Direct if correlated with symptoms	Rapid	Poor sensitivity and specificity; special skills are needed for interpretation
Culture	Can be performed in sophisticated clinical laboratory and in research laboratory	2–14 days	Definite; gold standard for most infection diagnosis	For phenotypic drug susceptibility testing	Time-consuming; poor sensitivity; limited microorganisms are culturable
Serology	Can be performed in larger and sophisticated clinical laboratory	4–6 h	Indirect	Automation	Results are generally retrospective; immunosuppressed host may be unable to mount a response
Molecular diagnostics	Can be performed in sophisticated clinical laboratory and in research laboratory	0.5–8 h	Direct without knowing microbial viability	High sensitivity and specificity	Quantification results awaits standardization; results might not be used as a test of cure

Significant changes have occurred in the field of diagnostic microbiology during the past 20 years. Until the early 1970s, definitive laboratory diagnoses of infectious diseases had been largely accomplished through the use of cumbersome, costly, time-consuming, and often subjective techniques. However, in the 1980s and 1990s, technologies evolved rapidly. For example, immunoassays came into wider usage with the development of radioimmunoassay and immunofluorescence, and then enzyme immunoassay (EIA) and immunoblotting, for routine diagnostic microbiology applications. These techniques, especially EIA, have supplanted, in many cases, the labor-intensive and relatively insensitive and nonspecific procedures such as complement fixation and hemagglutination inhibition assays for viral serology. Dependable, commercially available direct immunoassay kits now make detection of certain important groups of viruses economical, such as respiratory syncytial virus and rotavirus. Application of monoclonal antibodies and antigen-recognizing probes, such as peptides, has significantly increased the sensitivity and specificity of antigen detection.

Direct microorganism identification based on antigen or nucleic acid detection through immunologic and molecular techniques has significantly shortened the test turnaround time. Oligonucleotide probes play a significant role in culture confirmation and differentiation of slow-growing microorganisms, such as mycobacteria. Specialized nucleic acid hybridization probes, such as peptide nucleic acid (PNA) probes, are used for in-situ identification of organisms to the species level under a fluorescent microscope. Automated or semiautomated computerized commercial systems are now available for microorganism identification and antimicrobial susceptibility determination. Bacteremia and fungemia are now often detected by automated, instrument-assisted blood culture systems (see separate chapter), which have significantly improved the sensitivity and specificity for detecting most such pathogens and, by virtue of automation, have eliminated much of the hands-on time for detection of positive cultures.

Probably the greatest advance in the field of diagnostic microbiology has been in the area of nucleic acid probe detection. The polymerase chain reaction (PCR) and other recently-developed amplification techniques have simplified and accelerated the *in vitro* process of nucleic acid amplification. Rapid techniques of nucleic acid amplification and characterization, along with the increased use of automation and user-friendly software, have significantly broadened microbiologists' diagnostic arsenal. New real-time PCR, multiplex PCR, and microarray techniques provide relevant and accurate microbial quantification and typing which play important roles in the selection and monitoring of antimicrobial therapy.

Finally, advances in omics-based approaches (genomics, proteomics, and metabolomics) have led to their use in diagnostic microbiology and epidemiology. The development of robust next-generation sequencing platforms has dramatically increased sequencing capacity and reduced cost per base. These changes have made sequencing more accessible for the diagnostic laboratory such that sequencing is becoming a routine diagnostic tool in many laboratories. Mass spectrometry (MS)-based microbial identification and characterization are beginning to emerge in routine diagnostic services. The old diagnostic microbiology model, which was either labor intensive or required days to months before test results became available, largely serviced only a hospitalized patient population. The modern diagnostic microbiology laboratory has gradually begun servicing an increasing number of emergency room patients and outpatient populations, providing much more rapid results. Metabolomic profiles have also been shown to have clinical utility. Recent reports, for example, have shown that changes in serum metabolite profiles detected by nuclear magnetic resonance spectroscopy can be used to diagnose septic shock in pediatric patients and that the quantitative metabolomic profiles can be used to characterize sepsis severity and predictions of mortality (Mickiewicz et al., 2013).

Direct Identification of Microorganisms

Microscopic Examination

Many test specimens may be examined in their native state under a microscope. A wet mount can be prepared by applying specimens directly onto the surface of a slide, which is often used to detect motile trophozoites of parasites such as *Giardia lamblia* in stools and *Trichomonas vaginalis* in vaginal discharges. Certain bacteria are so thin that they cannot be resolved in direct preparations. Their characteristic motility, however, is an important feature of presumptive identification. Darkfield microscope examination, a method of allowing light to be reflected or refracted off the surface of the object, is used to identify these bacteria; spirochetes, such as *Borrelia burgdorferi*, the microorganism causing Lyme disease, are primarily visualized with this method. This method is also used for the demonstration of motile treponemes in exudates collected from a primary chancre of syphilis. Identification of characteristic cytoplasmic morulae in peripheral blood leukocytes is a timely way of confirming *Ehrlichia* infection. Detection of viral inclusions in smears or tissues has been the traditional means of directly demonstrating virus infections. Electron microscopy has been used directly from stool specimens for detection of the viral causes of gastroenteritis such as rotaviruses.

Examination of stained material, either direct from test specimens or samples of growth from cultures, is the most useful method for presumptive identification of several microorganisms. Microorganisms may be visualized and assigned to morphologic and functional groups using special stains. There are several staining methods that have been used in diagnostic microbiology (Table 2). The most popular is the Gram stain which is used to classify bacteria based on their forms, sizes, cellular morphologies, and color reactions. Other commonly used staining methods include the acid-fasting stain for mycobacteria, the acridine orange stain for cell wall-deficient bacteria (e.g., mycoplasma), the Giemsa stain for systemic protozoa (e.g., malaria), and iodine stains for intestinal helminthes. Calcofluor white stain binds to the cell walls of fungi in the presence of 10% potassium hydroxide, thereby increasing the sensitivity of direct fungus detection, including *Pneumocystis carinii*, a common opportunistic infection in AIDS and other immunocompromised hosts.

Microbial Antigen Testing

Immunological approaches to the detection of microorganisms' protein antigens remain important tools for the diagnosis and management of infectious diseases. Great technical advances have been made since the introduction of the early precipitation and agglutination assays. For immunologic detection of microbial antigens, latex particle agglutination, immunofluorescence assay, and EIA are the techniques most frequently used. Antibodies to a specific antigen are bound to latex particles to produce agglutination. This technique has been extremely useful in the detection of *Cryptococcus neoformans* in bodily fluid specimens, such as plasma and cerebrospinal fluid. Direct immunofluorescence assay has been included in the shell vial technique for rapid virus culture detection (see below) and the detection of *Legionella pneumophila* in sputum.

Table 2 Stains commonly used for detection of microorganisms

Stain method	*Organisms detected*	*Advantages*	*Disadvantages*
Gram stain	Bacteria, yeast	Rapid; direct differentiation; assess specimen for culture	Cell wall-deficient bacteria stain unpredictably; pink background often masks Gram-negative organisms
Acridine orange stain	Bacteria, *Mycoplasma*, plasmodia, *Babesia*	Good for organisms with damaged cell walls; background levels are low for structures that lack nucleic acid	Specific light source is needed; cannot differentiate bacterial Gram status reaction
Acid-fasting stain (Kinyoun or Ziehl-Neelsen)	Mycobacteria	Direct diagnosis of infection in untreated host	Cannot speciate mycobacteria; high background in tissue slide
Auramine-rhodamine stain	Mycobacteria	Lower power can be used for examining the slide	Fluorescence microscope is needed; artifact staining in tissue slide
Modified acid-fast stain	*Nocardia*, *Cryptosporidia*, *Isospora*, *Cyclospora*	Rapid and specific diagnosis	Tissue homogenates often mask presence of the organism
Calcofluor white stain with potassium hydroxide	*Pneumocystis*, fungi	Rapid stain for fungi detection	Fluorescence microscope with specific filter is needed; species differentiation requires skills
India ink stain	*Cryptococcus neoformans*	Diagnosis of meningitis when positive in spinal fluid	Low sensitivity; a messy technique with false positives from lymphocytes in hypotonic solution
Giemsa stain	Plasmodia, trypanosomes, leishmania, *Toxoplasma*, *Histoplasma*, *Pneumocystis*	Detection of multiple organisms; shows the relationship between organisms and host cells	Not specific for viral inclusions; cannot determine bacterial Gram status

There are several approaches to enzyme-linked antigen detection assays; the one most frequently designed for the detection of microbial antigens uses an antigen-specific antibody that is fixed to a solid-phase well in a plastic tray. Antigen present in the specimen binds to the antibody. The test is then completed by adding a second antigen-specific antibody bound to an enzyme that can react with a substrate to produce a colored product. A lateral flow chromatographic immunoassay (CIA) has recently been introduced and has been well received as it can rapidly detect several respiratory virus-specific antigens. In comparison to regular EIA procedures, these lateral flow CIA-based procedures require very little hands-on time and often are capable of a fast test turnaround time. Some of them require a hands-on time of only 1–2 min, have only one incubation period, and are very easy to interpret.

The use of microorganism-specific monoclonal and recombinant antibodies has improved reagent availability and reproducibility and enhanced test specificity. Examples include rapid viral antigen detection kits, which have been used widely in the clinical virology laboratory for the rapid detection of influenza virus, respiratory syncytial virus, and rotavirus in clinical specimens. To overcome the time-consuming and costly procedure for natural antibody production, phage display has been used to discover antigenic peptides, which are either chemically synthesized or produced in *E. coli*, and used to produce antibodies that can be probes for microbial antigen detection. They have been used for the detection of antigens of *E. coli* O157:H7, *Borrelia*, Epstein–Barr virus, and panels of biological threat agents.

Genetic Probe Hybridization

Nucleic acid probes are based on the detection of unique nucleotide sequences within the DNA or RNA of a microorganism; these unique nucleotide 'signatures' are surrogates for the presence of the organism itself. Bacterial ribosomes are highly conserved and essential organelles responsible for protein synthesis. The ribosomal RNA (rRNA) possesses distinct features that make it a good universal marker for bacterial identification. The oligonucleotide probes used to identify microorganisms are usually short sequences of DNA between 15 and 25 nucleotides long. Nucleic acid hybridization can be performed in liquid or solid formats. Different report molecules can be linked to the probes to visualize hybridization reactions.

Probe-based microbial detection is used in diagnostic microbiology primarily for culture confirmation of organisms after a brief period of *in vitro* cultivation. Gen-Probe, Inc. (San Diego, CA, USA) and Becton, Dickinson and Company (Sparks, MD, USA) make several culture identification nucleic acid probes that have been approved by the FDA. They produce rapid and reliable results for mycobacterial, fungal, and bacterial identification. Several products from AdvanDx Inc. (Woburn, WA) combining fluorescence *in situ* hybridization using PNA probes are available to directly identify common major blood culture isolates, including *Staphylococcus aureus*, Gram-negative bacilli as well as yeast pathogens, directly from positive blood cultures. The recently developed QuickFISH method allows for rapid detection and identification of pathogens from positive blood-culture bottles providing a total test-to-result time of less than 30 min (Tang and Peterson, 2014). Although these commercial products are more expensive than conventional approaches, the decrease in time-to-identification has the potential to improve patient outcomes and reduce overall health-care costs. A good example is to use such probes for speciation of cultured mycobacteria.

Microorganism Culture and Identification

Isolation and cultivation of a microorganism, either in an artificial medium or in a living host, is definitive evidence for the presence of a microbe. In many cases, culture techniques remain the 'gold standards' for diagnostic microbiology even though lengthy incubation periods preclude the use of the test results as useful diagnostic procedures. Culture is usually the most specific method for establishing the presence of a particular pathogen in a specimen. In addition, a pure culture of either a virus or a bacterium is essential for performing *in vitro* phenotypic antimicrobial susceptibility tests.

Culture Using Artificial Media

Bacteria, mycobacteria, mycoplasma, and fungi are cultured either in liquid or solid artificial media. Liquid media provide greater sensitivity for the isolation of small numbers of microorganisms since larger volumes of a specimen can be inoculated into broth than onto agar plates; however, liquid media cannot be used for diagnosis of mixed infections and organism quantification cannot be performed unless real-time growth indicators are measured. Solid agar media, on the other hand, though somewhat less sensitive than liquid media, provides isolated colonies that can be identified, sometimes based on their colony morphologies. For anaerobic pathogen recovery, media should be incubated in an anaerobic environment such as an anaerobic container or chamber. Bacteria present in specimens can also be quantified by calculating the number of colony-forming units on an agar plate.

Culture media can be made selective by incorporating compounds such as antimicrobial agents that inhibit endogenous flora, while permitting the growth of specific microorganisms resistant to these inhibitors. This is extremely important in the isolation and identification of pathogenic microorganisms in sputum and stool specimens. In the evaluation of respiratory tract infections in cystic fibrosis patients, for instance, several selective and differential media such as Hektoen enteric, xylose-lysine desoxycholate, and salmonella–shigella agars are available for the isolation and differentiation of enteric pathogens from stool samples. Bile-esculin agar plus vancomycin has been widely used as a selective and differential medium for screening vancomycin-resistant enterococci. In some instances, a growth indicator medium can be developed by incorporating one or more carbohydrates in the

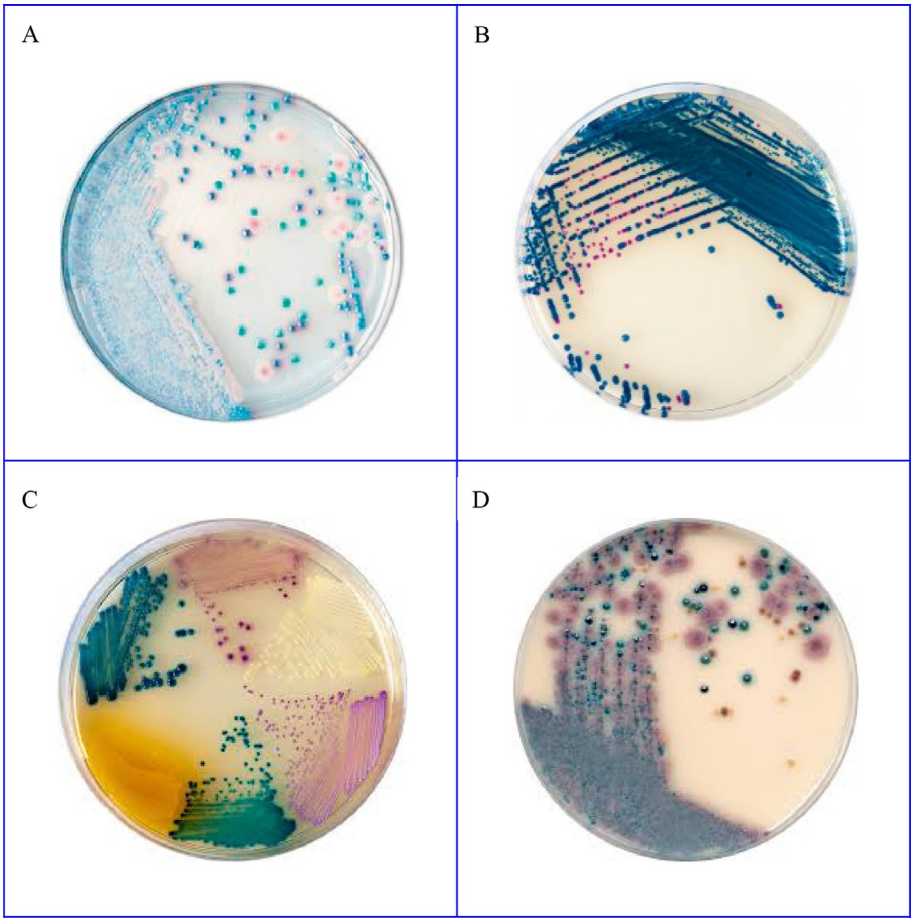


Figure 1 Screening for MRSA (methicillin-resistant *Staphylococcus aureus*) by using a chromogenic medium (a). A CHROMagar MRSA (Becton, Dickinson and Company) plate was inoculated with a nasal swab specimen and inoculated at 37 °C for 24 h. MRSA clones turned mauve while other bacterial colonies were inhibited, blue or colorless (BD, 2014). CHROMagar™ StrepB plate (b) is used to differentiate group B streptococci from other streptococci (CHROMagar, 2014b), CHROMagar ESBL (c) is used to select for and differentiate between Gram negative organisms producing extended-spectrum beta lactamase (CHROMagar, 2014a), and *Brilliance* Candida Agar (Oxoid) (D) differentiates between *Candida albicans* and *Candida tropicalis* from other species of *Candida* within 48 h (Oxoid, 2014).

medium along with a suitable pH indicator. These differential media can be used to isolate and screen for certain microorganisms. Many different chromogenic media have been developed to differentiate between bacterial genus and species and between yeast species (**Figure 1**). In addition, chromogenic media have been developed to screen for antibiotic – resistant organisms such as MRSA methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and Gram-negative organisms producing extended-spectrum beta lactamases. Isolation of microorganisms from blood specimens is a major task of clinical microbiology laboratories, and is described in detail in a separate article.

Culture Using Living Cells

Chlamydiae and many viruses can be recovered and identified in a eukaryotic cell culture system. The virologic component of diagnostic microbiology has become increasingly more common with the advent of effective antiviral treatments as well as the need to identify and treat viral infections in the human immunodeficiency virus (HIV)-infected and other immunocompromised hosts. Isolation is a very sensitive method because, theoretically, a positive result can be obtained with a single infectious virion. However, not all viruses can be recovered *in vitro*. Several culture systems have been used for virus isolation, and their characteristics are listed in **Table 3**. After inoculation, a virus can initially be identified and differentiated by (1) the pattern of the cytopathic effect (CPE), (2) the specific cells in which CPE is induced, and (3) the rapidity of the appearance of CPE. For example, the majority of enteroviruses cause CPE in rhesus monkey kidney cells within 48–72 h (Klespies et al., 1996; Lipson et al., 2001). However, poliovirus-induced CPE appears within 1 day after inoculation, whereas it takes 4 days for echovirus to induce the same amount of cellular destruction. Typically then, specimens are inoculated into as many different cell cultures as is reasonable to provide a susceptible host for each virus that may be present.

Table 3 Cell cultures used in diagnostic microbiology

Culture type	*Characteristic*	*Examples*	*Primary use*	*Remarks*
Primary	Diploid	Primary monkey kidney	Influenza, parainfluenza, enteroviruses	Endogenous virus infection, for example, SV-40; 1–2 passages only
Diploid cell lines	Diploid	Human diploid fibroblast MRC-5	Herpes simplex virus, cytomegalovirus, rhinovirus	Limited passage (50–70)
Established cell lines	Heteroploid	HEp-2, HeLa	Respiratory syncytial virus, adenovirus	Continuous passage; mycoplasma contamination

Shell vial isolation, which combines cell culture and antigen detection, has been adapted for the detection of several viruses as well as chlamydia. This method represents a relatively rapid means of identifying agents whose identifications may ordinarily take as long as 3 weeks in a traditional tube culture. Vials with coverslips are typically seeded with a monolayer of cells in growth medium. When the cells are nearly confluent, the growth medium is aspirated, and then a certain amount of specimen is added. The vials are centrifuged, after which growth medium is added. The vials are incubated for specific times and temperatures (depending on the agent being sought), and the cells on the coverslip are then incubated with fluorescein-conjugated virus-specific antibody directly in the shell vial. The stained coverslip is removed and placed on a slide for examination under the fluorescence microscope. This technique is used especially for the detection of cytomegalovirus (CMV) infections, since CMV-induced CPE is relatively slow to develop. A genetically altered cell line, the enzyme-linked virus inducible system (Diagnostic Hybrids, Inc., Athens, OH, USA), has been widely used to rapidly detect herpes simplex viruses in cell culture. Transgenic and cocultured cell lines have enhanced the sensitivity of recovery of enteroviruses and eliminate the need for multiple shell vials. Mixed cell lines stably transfected with host cofactors involved in the binding and entry of some enteroviruses (Super E-Mix) are commercially available (Diagnostic Hybrids, Inc.). These mixed cell cultures have been shown to be more sensitive for virus recovery from clinical samples compared to conventional tube or shell vial cultures with single cell lines.

Culture Using Other Living Hosts

Microorganism isolation, especially for some viruses, occasionally requires the inoculation of test specimens into animals and embryonated eggs. Several arboviruses that cause encephalitis can be isolated by inoculating the implicated specimen into suckling mice. Embryonated eggs are still the best culture media for influenza viruses. Susceptible animal inoculation, more recently into severe combined immunodeficiency mice and other rodents, has been a powerful tool to hunt for a variety of unknown pathogens. *L. pneumophila*, the bacterial organism causing Legionnaires' disease, was first identified by inoculating the clinical materials into susceptible guinea pigs.

Measurement of Host Responses to Infection

Instead of detecting the presence of the microorganism, serologic tests measure the host's humoral immune response to a microorganism infection. Serologic tests are, in certain circumstances, the mainstay for diagnosis of certain infections whenever recovery of those organisms in culture is difficult or impossible. However, there is usually a lag between the onset of microorganism infection and the development of antibody to the organism. Although IgM antibody appears relatively rapidly, IgM testing may be subject to interference by rheumatoid factors. For IgG testing, it is often necessary to obtain paired serum specimens, one taken during the acute phase of the disease and one taken during convalescence, to look for a rising titer of IgG antibody. Detuned antibody assays determine antibody avidity by diluting or treating tested serum specimens, thereby allowing the distinction between recent and distant infections. This method has been used mainly in HIV clinical research for epidemiology, surveillance, and clinical investigations. Another limitation on the use of serology as a diagnostic tool is that immunosuppressed hosts may be unable to mount an antibody response. In general, however, detection of specific antibody responses can be a very useful diagnostic approach in the post-acute setting where causative organisms may be rare or completely absent. For example, serological testing for Lyme disease remains the mainstay of diagnostic evaluations because at the time many patients present, organisms have disseminated from the site of infection and are difficult or impossible to detect by direct methods based on culture or PCR.

Immunofluorescence Methods

Immunofluorescence techniques have been widely used in the past for detection of microorganism-specific antibodies and still have many applications. The common procedure used in a clinical serology laboratory is an indirect one. If present, the antibody in the serum specimen reacts with the microorganism-specific antigen, which was prefixed on a glass slide. After a wash step, fluorescein-conjugated immunoglobulin directed against the test serum species is overlaid on the slide. After washing, the slide

is examined for specific fluorescence under a fluorescence microscope. This technique is still the primary choice for diagnosis of several virus infections, including Epstein–Barr virus and measles virus, in the majority of clinical virology or serology laboratories. However, fluorescence techniques are quite labor intensive and interpretation of the results is subjective; these techniques have, therefore, been gradually replaced by automatable and more objective serologic techniques such as EIAs.

Enzyme Immunoassays

EIAs have replaced radioimmunoassays in most diagnostic applications, and have become among the most widely used diagnostic methods in a clinical serology laboratory. In its most used format, the solid-phase EIA technique uses plastic microtiter plates or beads to which antigens are passively adsorbed. In a noncompetitive procedure, a test serum specimen is added. If present, the microorganism-specific antibody binds to the antigen. After washing, an enzyme-labeled immunoglobulin specific for the test serum species is added. After another wash step, a chromogenic enzyme substrate is added, and the color that develops is proportional to the amount of specific antibody present. The application of EIA serologic techniques for diagnosis of viral and ricketsial infections has expanded significantly. This assay is most frequently used in screening for immune status, such as in rubella testing, but they are also used as primary screening tests for infections due to HIV, HCV, HBV, and human T-cell lymphotrophic virus.

Immunoblotting

The specificity of a serologic test is largely determined by the antigen used to capture the antibody in the direct test formats. An immunoblot (or Western blot) is one of the most specific serologic methods available. The general components of immunoblotting as used for immunodiagnosis with human sera include (1) electrophoretic separation of protein antigen on sodium dodecyl sulfate polyacrylamide gels, (2) electrophoretic transfer of the protein bands to a nitrocellulose or other support membrane, (3) blocking of free protein-binding sites on the membrane, (4) addition of the test serum, and (5) detection of the specifically bound serum antibodies. It is then possible to determine whether the patient's antibodies are directed against pathogen-specific or cross-reactive antigens. This additional level of analysis allows the discrimination of specific and nonspecific reactions. Immunoblotting has become a mainstay for confirming infection with HIV, HCV, and *B. burgdorferi* after initial positive EIA results.

Other Serologic Techniques

Other methods to detect immune humoral response to microorganism infections include agglutination and complement fixation assays. Agglutination reactions can be defined as the specific immunochemical aggregation of polystyrene (latex) particles coated with microorganism antigens that can be used to detect antigen-specific antibodies. Agglutination assays are simple to perform, and require only 15–30 min for completion, offering a useful alternative to EIA. This test has been used in the clinical serology laboratory for detection of rubella virus antibodies and quantification of rapid plasma reagin, which can be used as an indication of the therapeutic efficacy of syphilis treatment. Although the complement fixation test is still used in the serodiagnosis of several fungal infections, the system is extremely labor intensive, relatively insensitive, and will be gradually replaced with other formats. New techniques and formats in antibody testing have been emerging. A multianalyte profile (xMAP) allows simultaneous detection and differentiation of a panel of antibodies in a single reaction by using xMAP multiplexed technology (Luminex, Austin, TX, USA), which is highly desirable in clinical laboratories to provide a differential diagnosis of nonspecific conditions.

Other Host-Response Determining Techniques

In addition to humoral immune responses, other host responses have been used in diagnostic microbiology field. The QuantiFERON-TB Gold (Cellestis, Carnegie, Australia) and the T-SPOT.TB (Oxford Immunotec, Oxford, UK) tests were approved by the US FDA in 2001 and 2008, respectively, to measure host cell-mediated immune reactivity to *M. tuberculosis* as an aid for detecting latent tuberculosis infection. Both tests are based on the quantification of interferon-γ released from sensitized lymphocytes in whole blood incubated overnight with purified protein derivative from *M. tuberculosis* and control antigens.

C-reactive protein (CRP) is pentameric protein that aids in non-specific host defense against inflammatory processes and cellular breakdown products by activating the classical complement cascade. CRP is one of the most sensitive acute-phase measures of inflammation, with concentrations rising up to 1000-fold over baseline levels after such events as infection, trauma, surgery, burns, tissue infarction, and advanced cancer. Concentrations of CRP are generally higher in patients with bacterial infections relative to those with viral infections. However, high CRP levels may also be observed in individuals with influenza or mononucleosis. Overall, changes in CRP concentrations provide useful diagnostic information about the acuity and severity of a disease as the increase in CRP with inflammation occurs within 6–12 h and peaks at 48 h. However, caution must be taken when interpreting CRP results, as CRP elevations are non-specific and the magnitude of CRP levels may be less pronounced in patients with liver disease (Gabay and Kushner, 1999).

Procalcitonin (PCT), the biologically active precursor of the calcium-modulating hormone calcitonin, has been shown to be a biomarker for bacterial infection (Simon et al., 2008). PCT has clinical utility for diagnosis of bacteremia, sepsis, differentiating

viral from bacterial infections, and diagnosis of systemic secondary infection post-surgery, in severe trauma, burns, and multi-organ failure. PCT is strongly correlated with severity of infection as levels of PCT increase significantly as patients progress from sepsis to severe sepsis and septic shock (Barati et al., 2008; Harbarth et al., 2001; Wanner et al., 2000). PCT has been investigated for use in decisions regarding antibiotic therapy de-escalation and discontinuation; with a half-life of 24–35 h, PCT is also a good candidate for monitoring treatment progress. Studies have shown that monitoring serial PCT levels to guide the duration of antimicrobial therapy substantially reduces antimicrobial use and patient length of stay in the ICU without compromising clinical outcomes (Agarwal and Schwartz, 2011; Simon et al., 2008). PCT has been shown to be a more sensitive indicator over CRP to different infectious versus non-infectious causes of inflammation as noninfectious inflammatory stimuli need to be extremely severe to result in PCT elevations. Finally, cancer patients undergoing chemotherapy often have periods of neutropenia and a weakened immune response to bacterial infections. For this reason, PCT levels in this population do not stratify according to infection severity and, therefore, the clinical relevance of PCT in this population is unclear (de Bont et al., 2000).

Molecular Techniques in Diagnostic Microbiology

Identification methods based on biochemical phenotypic parameters can, in some cases, result in confusing or misleading results, especially when the number of features that can be analyzed is limited. Over the past 10 years, nucleic acid amplification technology has opened new avenues for the detection, identification, and characterization of pathogenic organisms in diagnostic microbiology. Molecular applications enhance the speed, sensitivity, and sometimes the specificity of an etiologic diagnosis. One good example is the detection and identification of pathogenic organisms directly from positive blood cultures. The promise of these techniques is the replacement of biological amplification – growth in culture – by enzymatic amplification of specific nucleic acid sequences, which is extremely useful to identify microorganisms that cannot be cultivated. The disadvantage, on the other hand, is the lack of recovered isolates for antimicrobial resistance determining. A full molecular procedure consists of specimen processing, nucleic acid amplification, and amplification product identification. Nucleic acid amplification techniques fall into three general amplification categories, which all share certain advantages over traditional methods, particularly for the detection of fastidious, unculturable, and/or highly contagious organisms (**Table 4**).

Table 4 Nucleic acid amplification methods

Amplification method	*Amplification category*	*Manufacturer (Trade mark name)*	*Enzymes used*	*Temperature requirement*	*Nucleic acid target*
Polymerase chain reaction	Target	Roche Molecular System, Inc. (Amplicor, Cobas); Abbott Molecular (m2000); Cepheid (GeneXpert); Qiagen (Rotor-Gene Q MDx); Quidel (QuantStudio™ Dx), etc.	*Taq* DNA polymerase	Thermal cycler	DNA (RNA)
Transcription-mediated amplification	Target	Gen-Probe, Inc. (Aptima)	Reverse transcriptase, RNA polymerase, RNase H	Isothermal	RNA or DNA
Nucleic acid sequence-based amplification	Target	bioMerieux (NucliSens)	Reverse transcriptase, RNA polymerase, RNase H	Isothermal	RNA or DNA
Strand displacement amplification	Target	Becton, Dickinson and Company (ProbeTec)	Restrictive endonucleonase, DNA polymerase	Isothermal	DNA
Helicase-dependent amplification	Target	Quidel/BioHelix Corp. (IsoAmp)	Helicase, *Taq* DNA polymerase	Isothermal	DNA (RNA)
Cycling probe technology	Probe	ID Biomedical, Vancouver, BC, Canada (CPT-strip)	RNase H	Isothermal	DNA (RNA)
Ligase chain reaction	Probe	Abbott Laboratories, Abbott Park, IL, USA (LCx)	DNA ligase	Thermal cycler	DNA
Hybrid capture system	Signal	Qiagen/Digene (Hybrid Capture)	None	Isothermal	DNA
Branched DNA	Signal	Siemens/Bayer Diagnostics, Emeryville, CA, USA (Versant)	None	Isothermal	DNA or RNA
Invader technology	Signal	Hologic/Third Wave Technologies (Invader)	Cleavase enzyme	Isothermal	DNA

Nucleic Acid Extraction

A good specimen preparation is comprised of efficient target recovery, establishment of the integrity of nucleic acid targets, optimal removal of amplification inhibitors, elimination of components that affect other enzymatic substrates, and sterilization of potentially hazardous organisms. Numerous commercial methods and kits that are manual or automatic, with low or high throughput, are available for the extraction of microbial nucleic acids from a variety of clinical specimens. QIAamp MinElute Virus Kits (Qiagen, Inc., Valencia, CA, USA) have been demonstrated to effectively extract viral total nucleic acids from cerebrospinal fluid and respiratory specimens. Several clinical services have recently switched to use of the easyMAG platform (bioMerieux, Durham, NC, USA) to take advantages of its automation, flexibility, and yield of high quality and quantity of total nucleic acids.

In Vitro Nucleic Acid Amplification Systems

According to the theoretical basis, *in vitro* nucleic acid amplification techniques can be placed into one of three broad categories, which all share certain advantages over traditional methods, particularly for the detection of fastidious, unculturable, and/or highly contagious organisms (Table 4). The first category is a target amplification system, which includes PCR, transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), and helicase-dependent amplification (HDA), where many copies of the nucleic acid target are made. Among these amplification systems, PCR and PCR-derived techniques are the best developed and most widely used methods of nucleic acid amplification (for principles of PCR, see separate article) in the clinical setting. Semiautomated and automated systems have now been manufactured by Roche Molecular System, Inc. (Indianapolis, IN, USA), Abbott, Cepheid, Becton Dickinson and other companies for detection and/or quantification of several organisms including HIV, HCV, HBV, CMV, HSV, HPV, MRSA/*S. aureus*, *Clostridium difficile*, enterovirus, influenza, respiratory syncytial virus, group B Streptococcus, vancomycin-resistant enterococci, norovirus, *C. trachomatis*/*N. gonorrhoeae*, *Mycobacterium tuberculosis* and a variety of other organisms. In addition, numerous user-developed PCR-based DNA amplification techniques have been developed and applied to the detection of microbial pathogens, identification of clinical isolates, determination of antibiotic resistance, and strain subtyping. Commercially available products based on TMA, NASBA, HDA, and SDA are available from Gen-Probe, Inc., bioMerieux, BioHelix Corp. (Beverly, MA, USA), and Becton Dickinson and Company to detect and quantitate several microorganisms including *M. tuberculosis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, MRSA, *C. difficile*, HIV, HCV, and enteroviruses (Table 4).

A second approach is probe amplification in which many copies of the probe that detects the target nucleic acid amplified after target-specific hybridization occurs. The ligase chain reaction (LCR) was the most widespread application of probe amplification in diagnostic microbiology until its withdrawal from the market (Table 4). When used following a target amplification method, such as PCR, LCR can be sensitive and useful for the detection of point mutations. Although convenient and readily automated, the main drawback of LCR was the difficult inactivation of postamplification products. The nature of the technique does not allow for the most widely used contamination control methods to be applied. Another technique in the system, cycling probe technology (CPT), which incorporates a unique chimeric DNA–RNA–DNA probe, has been designed to detect a specific sequence with *mecA* and *vanA*/*B* genes for diagnostic use as a culture confirmation assay for MRSA and vancomycin-resistant enterococci.

The last amplification system is signal amplification, which is designed to strengthen a signal by increasing the concentration of label attached to the target nucleic acid. Unlike procedures which increase the concentration of the probe or target, signal amplification increases the signal generated by a fixed amount of probe hybridized to a fixed amount of specific target. The fact that signal amplification procedures do not involve a nucleic acid target or probe amplification is a theoretical advantage, because of lower susceptibility to contamination problems inherent in enzyme-catalyzed nucleic acid amplification. Sensitivity, however, compared to target nucleic acid amplification techniques, may be a limiting factor. Another limitation of signal amplification is background noise due to the nonspecific binding of reporter probes. Currently, three diagnostic companies have their signal amplification products available for molecular diagnostic purposes. The Digene Hybrid Capture System (Digene Diagnostics, Inc., Gaithersburg, MD, USA) is widely used to determine HPV infection in cervical swabs. Homogeneous Invader Technology (Hologic/Third Wave Technologies, Madison, WI, USA) is another signal amplification system, which is a powerful tool for genetic analysis of single nucleotide polymorphisms in both microorganisms and hosts.

Detection and Identification of Amplification Products

After amplification, the millions of target DNA copies that accumulate are invisible to the naked eye; therefore, an appropriate detection method is needed to 'visualize' amplified DNA molecules. On the other hand, an additional identification system is critical to enhance both sensitivity and specificity of the PCR amplification procedure. Detection and identification of amplification products, or amplicons, has become a routine procedure in the molecular diagnostic laboratory. The classical means of detection and characterization of the PCR product was agarose gel electrophoresis, where sensitivity can be enhanced by Southern blot hybridization using DNA probes. Several user-friendly and rapid techniques have been adopted by molecular diagnostic laboratories, including direct sequencing, nucleotide microarray, mass spectrometry, and a 'real-time' procedure. Due to the advancement of gene sequencing techniques, gene sequencing has become a powerful tool for molecular diagnostic laboratories. High throughput sequencing has reduced the cost and increased output for laboratories to identify and differentiate between closely-related bacteria and viruses. For example, gene sequencing is used to monitor HIV-1 responses to therapy and to identify genomic

antiviral drug resistance mutations. In addition, gene sequencing has been applied to *Mycobacterial* spp. identification. The 16S rRNA gene has traditionally been the most frequently used target for differentiation of *Mycobacterium* species. However, the 65-kDa heat-shock protein (*hsp65*) has been shown to have more inter-species variability than the 16S rRNA gene and is, therefore, more useful for differentiating between closely-related species (Kapur et al., 1995; McNabb et al., 2004). Microarray technology is becoming more accessible for pathogen detection in the clinical setting. New devices are being developed that automate DNA extraction from patient specimens, labeling, and scanning. In one example, the preliminary array technology included 11 different Gram negative, Gram positive, and yeast pathogens. These arrays successfully identified organisms collected from infected wound swab samples (Palka-Santini et al., 2009).

Abbott Molecular has released the PLEX-ID which combines multi-locus broad-range PCR amplification followed by electrospray ionization-quadropole mass spectrometry (PCR-ESI-MS) to identify microbial pathogens. In this system, multiple PCR primers are used to amplify highly-conserved regions of bacterial, viral, or yeast genomes. The PCR amplicons are subsequently analyzed by the ESI MS and compared to the IBIS Bioscience database (Abbott Molecular) containing information on the identity of over 750 000 organisms. Further studies are needed to validate the usefulness of this technology for a greater number and diversity of pathogens; however, preliminary results are promising for the successful identification an expansive diversity of pathogens (Drake et al., 2011; Fournier et al., 2013).

Arguably the biggest advance in PCR technology has been the development of closed system detection of amplification products which obviates the need for contamination-prone removal of amplification products. Early applications of PCR comprised PCR amplification followed by a separate identification of PCR products. With the introduction of a fluorescence resonance energy transfer, nucleic acid amplification, detection, and probe-specific confirmation of PCR products, no transfers of PCR products out of the reaction tube are required. Real-time PCR is closed' and, therefore, avoids the important concerns of carryover contamination with previous amplification products. As a result of shorter thermocycling and simultaneous product detection, the turnaround time for results has also been significantly shortened. This technique is widely used to detect and quantify microorganisms and determine mutations in both microorganism and host genes. Real-time detection and characterization is also available in other *in vitro* amplification systems such as the Invader and NASBA systems.

Non-Genomic Molecular Testing

Detection of Metabolic Products

The causal relationship between *Helicobacter pylori* colonization of the gastric mucosa and gastritis was first proposed in 1983 by Warren and Marshall, who received the Nobel Prize in Physiology or Medicine in 2005. *H. pylori* produces large amounts of extracellular urease which hydrolyzes urea to form ammonia and soluble carbon dioxide, which can be detected within hours in human breath specimens. The noninvasive urea breath test has enabled the direct detection of *H. pylori* infections without endoscopy. However, the urea breath test was not widely accepted in the routine clinical microbiology laboratory until a commercial, nonradioactive 13C-based kit (Meretek Diagnostics, Lafayette, CO, USA) was approved by the US Food and Drug Administration (FDA) in 1996. The urea breath test has excellent sensitivity and specificity for the initial diagnosis of active infection in untreated patients and for treatment follow-up after therapy. The test is noninvasive and highly accurate, and newer assay formats and instruments are much simpler, more cost effective, and more user friendly. The test has been recommended as the method of choice to establish or exclude *H. pylori* infection without endoscopy. Volatile organic compounds (VOC) have also shown promise as a diagnostic breath test for *Aspergillus* as unique VOCs are produced by five different *Aspergillus* species. Further, detection of the VOCs 2-aminoacetophenone and nicotinic acid has shown promise as POC tests for *Pseudomonas aeruginosa and Mycobacterium tuberculosis* infections, respectively (Wang et al., 2013; Zhu et al., 2010).

Microbial Identification by Mass Profiling

Few developments in microbiological diagnostics have revolutionized the routine identification of microorganisms in clinical microbiology laboratories as matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS has been widely applied as an easy, rapid, high-throughput, low-cost, and efficient technique for the phenotypic characterization of Gram-positive and -negative bacterial whole cells, simple cell lysates, or bacterial products such as lipopolysaccharides and proteins. Investigations are currently under way to use MALDI-TOF MS for microbial subspecies typing and identification, with attention being made regarding the phenotypic detection of certain antibiotic resistance mechanisms, e.g., β-lactamases and carbapenemases (Wieser et al., 2012). The cost of MALDI-TOF MS instruments is high (hundreds of thousands of dollars per instrument); however, the cost per sample is low (approximately $1 per sample) (Chen et al., 2013).

Early attempts to use MALDI-TOF MS for pathogen identification directly from positive blood cultures failed due to patients' blood proteins interfering with the analysis. However, protocols have since been developed that separate the bacterial cells from host proteins through cell lysis or differential centrifugation steps. With the additional sample processing, correct species-level identification can be achieved in 75–95% of tests. Similarly, the routine identification by MALDI-TOF MS of yeast isolates is reliable and much quicker than conventional techniques. Identification of fungal samples by MALDI-TOF MS is more complicated due to the various growth forms of cultured molds, such as mycelium and conidia, which obscure the analysis due to their different protein compositions.

Transcriptomics

Efforts to develop strategies for eradication of HIV-1 have intensified in recent years. However, while $CD4^+$ T cell count and plasma viral load have been important markers of disease progression, their reliability has recently come into question as these markers do not reliably identify individuals on anti-retroviral medication with virological failure (Moore et al., 2008). Therefore, novel biomarkers of disease detection and progression are needed. Recent reports have identified levels of miR-150 and miR-146b-5p in peripheral blood mononuclear cell and plasma as predictive of HIV/AIDS disease progression and therapy (Munshi et al., 2014).

In the case of *Mycobacterium tuberculosis* infections, effective treatment regimens rapidly decrease the number of viable *M. tuberculosis* organisms in sputum, with the number of cultivatable bacilli typically reduced by approximately 10-fold within the first 1–2 weeks. However, because of the slow growth rate of *M. tuberculosis*, results of cultures for samples obtained early in treatment to monitor treatment efficacy will not be available in a timely manner (Mdivani et al., 2009). Bacterial clearance in TB patients receiving chemotherapy is important for a number of reasons, including early diagnosis of treatment efficacy, prevention of relapse due to incomplete cure, and as endpoints in clinical trials evaluating new tuberculosis medications (Bertholet et al., 2011). As a consequence, an mRNA RT-PCR assay analyzing antigen 85B mRNA from sputum samples has been developed as a test-of-cure for *M. tuberculosis* infections. This assay allows real-time, rapid monitoring of the response to anti-TB therapy and has a sensitivity and specificity of 85.2% and 88.6%, respectively. The antigen 85B mRNA RT-PCR assay data correlated clinically with resistance to anti-TB drugs, whereas the DNA PCR assay showed a high false-positive rate (Bertholet et al., 2011).

Molecular Diagnosis and Monitoring

Detection and Identification of Microorganisms

Over the past century, the identification of microorganisms has relied principally upon phenotypic characteristics such as morphology and biochemical characterization. Genotypic identification has emerged as an alternative or complement to established phenotypic methods. Broad-range PCR primers are designed to recognize conserved sequences in the phylogenetically informative gene of a variety of bacteria, and highly variable regions between the primer binding sites are amplified by PCR. The amplified segment is sequenced and compared with known databases to identify a close relative. The growing availability of whole-genome bacterial sequences has enabled the selection of PCR targets to identify organisms, detect virulence factors, antibiotic resistance markers, and for molecular genotyping.

The most successful example is the ribosomal rRNA gene sequence-based microbial identification system, which includes the extraction of nucleic acids, PCR-mediated gene amplification, sequence determination, and computer-aided analysis. Multi-locus sequence typing (MLST) compares a set of unique loci to a database to identify pathogens. Using this method, virulence factors and antibiotic resistance markers can also be easily identified. The time, effort, and cost associated with sequencing data analysis were previously major limitations of using sequencing technologies in a clinical setting. However, release of technologies such as Ion PGM (Life Technologies) and the MiSeq (Illumina) have decreased sequencing costs while simultaneously increasing efficiency and improving turnaround times (Didelot et al., 2012).

Microorganism Quantification

Quantification of nucleic acid targets has become desirable in the clinical practice to monitor therapeutic responses and provide prognostic information for infectious diseases. The task of quantitative amplification has been problematic since the amplification techniques yield products in an exponential manner until a plateau is reached; any factor interfering with the exponential nature of the amplification process would thereby affect the result of the quantitative assay. To overcome this problem, a competitive real-time PCR assay was developed based on coamplification of an internal competitor with the target sequence. Currently, quantification of microorganism load is based primarily on (1) target amplification, which includes both PCR- and NASBA-based techniques, and (2) branched DNA-based signal amplification. Commercially available kits are available to make it possible for many clinical laboratories to carry out quantitative analysis. Real-time PCR-based systems represent a significant advance and are widely being used for microorganism quantification in clinical microbiology services. The unique characteristic, simultaneous, cycle-by-cycle detection of PCR products has made possible a rapid, high-throughput quantification of nucleic acid sequences. Several real-time PCR systems have been successfully used to manage infections caused by HBV, HCV, HIV, CMV, Epstein–Barr virus, and BK virus.

Multiplex Testing

Molecular screening of 'at risk' populations for a group of possible pathogens is an exciting area of development in molecular microbiology. Multiplex PCR, which utilizes numerous primers within a single reaction tube in order to amplify nucleic acid fragments from different targets, can screen a clinical specimen for several probable pathogens. Modifications have been made to improve the conventional multiplex PCR procedure.

For example, clinical presentations for the majority of respiratory tract infections are often nonspecific. Traditionally, different methods of detection are employed for each group of pathogens. This requires special media, equipment, and expensive facilities for the culture of mycobacteria, virology facilities, and special media for *Legionella*, *Chlamydia*, and *Mycoplasma*. Although nucleic

acid-based diagnostics have emerged as the new gold standard, large numbers of potential etiologies of respiratory infections limit detection efficiency by using multiple, single-pathogen specific, molecular assays. Several multiplex-PCR-based devices are commercially available for detection and simultaneous identification of a panel of respiratory or gastrointestinal pathogens, respectively. For example, the xTAG Gastrointestinal Pathogen Panel (xTAG GPP, Luminex, Austin, TX) was the first FDA-cleared device to detect 11 major gastrointestinal pathogens (Dunbar et al., 2013). BioFire has released the Filmarray with respiratory (17 viruses, 3 bacteria), blood pathogen (16 bacteria, 5 yeast, 3 resistance marker genes), and GI (13 bacteria, 5 viruses, 4 parasites) panels. The FilmArray instrument integrates sample preparation, amplification, detection and analysis into one simple system that requires approximately 2 min of hands-on time and has a total run time of about 1 h. Similar devices are available from Seegene (Rockville, MD) and GenMark (Carlsbad, CA).

Rapid, On-Demand, and Point-of-Care Testing

Rapid provision of results can facilitate better clinical decision-making, improved patient adherence, and greater patient satisfaction, all of which lead to improved clinical outcomes with wider patient, operational, economic, and societal benefits. For example, early and accurate diagnosis of the central nervous system infections has been critical to clinical intervention in terms of choosing chemotherapy and predicting prognosis. It has been demonstrated that early diagnoses of enterovirus-related meningitis improve the management and decrease the costs of care by reducing hospital stay length and antibiotic use. Similarly, direct detection of *Streptococcus pyogenes* antigens in throat swabs can improve the treatment of pharyngitis by detecting the majority of streptococcal infections while the patient is still in the office. Detection of specific nucleic acids is more sensitive but also less rapid and more expensive than antigen detection.

Technology advances in molecular microbiology now enable a wide range of diagnostic tests to be provided at the point of care. The GeneXpert Dx diagnostic system (Cepheid) is an important new development in the field of molecular diagnostics. It automates all of nucleic acid diagnostic steps in a disposable, microfluidic cartridge and is the first real-time PCR system that can be used to provide 'real-time service.' Although it was approved by the FDA as a moderately complex test, it is simple enough to be performed reliably by individuals without a background in nucleic acid diagnostics. Sample preparation reagents and samples are all that are required to add to the cartridge by the user. It has independently controlled and operated analysis modules that facilitate testing of individual samples in a random-access mode (**Figure 2(a)**). The complete automation and rapid result capability of the GeneXpert make it uniquely suited for on-demand and point-of-care testing. The Alere™ i (**Figure 2(b)**) is a small isothermal nucleic acid amplification platform that allows for the qualitative point-of-care detection of infectious diseases. For example, the Alere™ i Influenza A & B panel produces flu results in less than 15 making it faster than other molecular methods and more accurate than conventional rapid tests. The IQuum Liat™ Analyzer (**Figure 2(c)**) automates all nucleic acid testing processes, including reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, amplification and real-time detection. The machine has a very small foot print and is built with a touch screen, eliminating the need for an additional neighboring computer, and reports results in 20–60 min depending on the test. IQuum is currently developing additional assays for HIV, CMV, Influenza subtyping, and dengue diagnostics to use with the Liat™ platform. Quidel/Biohelix has developed several Helicase-dependent isothermal amplification diagnostic assays (AmpliVue series) (**Figure 2(d)**). These assays do not require any instrument and can be performed at room temperature. Quidel currently has AmpliVue assays available for *C. difficile*, group A and B streptococci, and HSV 1 and 2.

Laboratory Automation

Technological advances have brought on more fully automated devices for the busy clinical microbiology laboratory. BD Diagnostics offers a portfolio of total automated instrumentation technologies to automate manual and time-consuming tasks (**Figure 3(a)**). The Previ Isola (BioMerieux) (**Figure 3(b)**) is an automated plate streaker and specimen processing instrument. The Previ Isola plates, streaks, and labels as many as 180 plates per hour. Copan has recently partnered with Siemens and Thermo Fisher Scientific to distribute the Walk-Away Specimen Processor (WASP) (**Figure 3(c)**) which is a comprehensive system which encompasses all aspects of liquid specimen processing including plate streaking, gram slide preparation, and enrichment broth inoculation. The Sirscan2000automatic by i2a incubates samples and reads and publishes results within 4–7 h (**Figure 3(d)**).

Concluding Remarks

Diagnostic microbiology has been developing new methods to rapidly detect and accurately identify implicated microorganisms in test specimens through a variety of techniques. Technologic changes have made significant progress in the various areas including bacteriology, mycology, mycobacteriology, parasitology, and virology during the last two decades in the field of diagnostic microbiology. The physical structure of laboratories, staffing patterns, work flow, and turnaround time have all been influenced profoundly by these technical advances. The implementation of nucleic acid amplification-based molecular techniques provides complementary, rapid, and on-demand diagnosis services. These changes will continue, and lead diagnostic microbiology inevitably to be a modern discipline, which will be equipped to face inevitable challenges in the future.

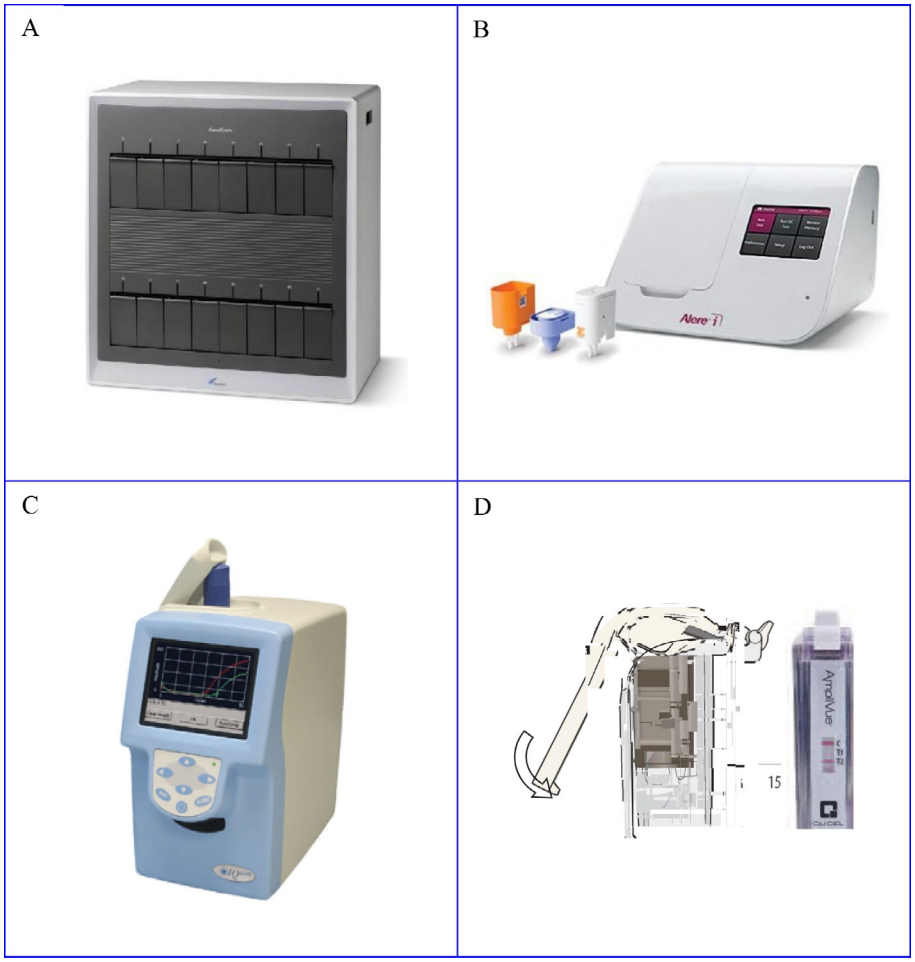


Figure 2 Rapid, on-demand, and point-of-care testing. The Xpert EV assay (Cepheid) comes as a unit dose, self-contained kit (Xpert enterovirus self-contained cartridge) run on the GeneXpert Dx diagnostic system (a) (Cepheid, 2014). The only extra equipment required is a pipette that can deliver 140 ml of Lysis Reagent and sample into the cartridge. The whole test takes less than 5 min of hands-on time, and results are available in about 2.5 h. The Alere™ i Influenza A & B panel (b) is a point-of-care tests that offers results in as few as 15 min (Quidel, 2014). The IQuum Liat Analyzer™ (c) automates molecular testing for influenza A and B (Iquum, 2011). The machine has a small footprint and 20–60 min turn-around time enabling its use for point-of-care diagnostics. The Quidel AmpliVue kits (d) are available for instrument-independent, room-temperature molecular diagnostics (Alere, 2014).

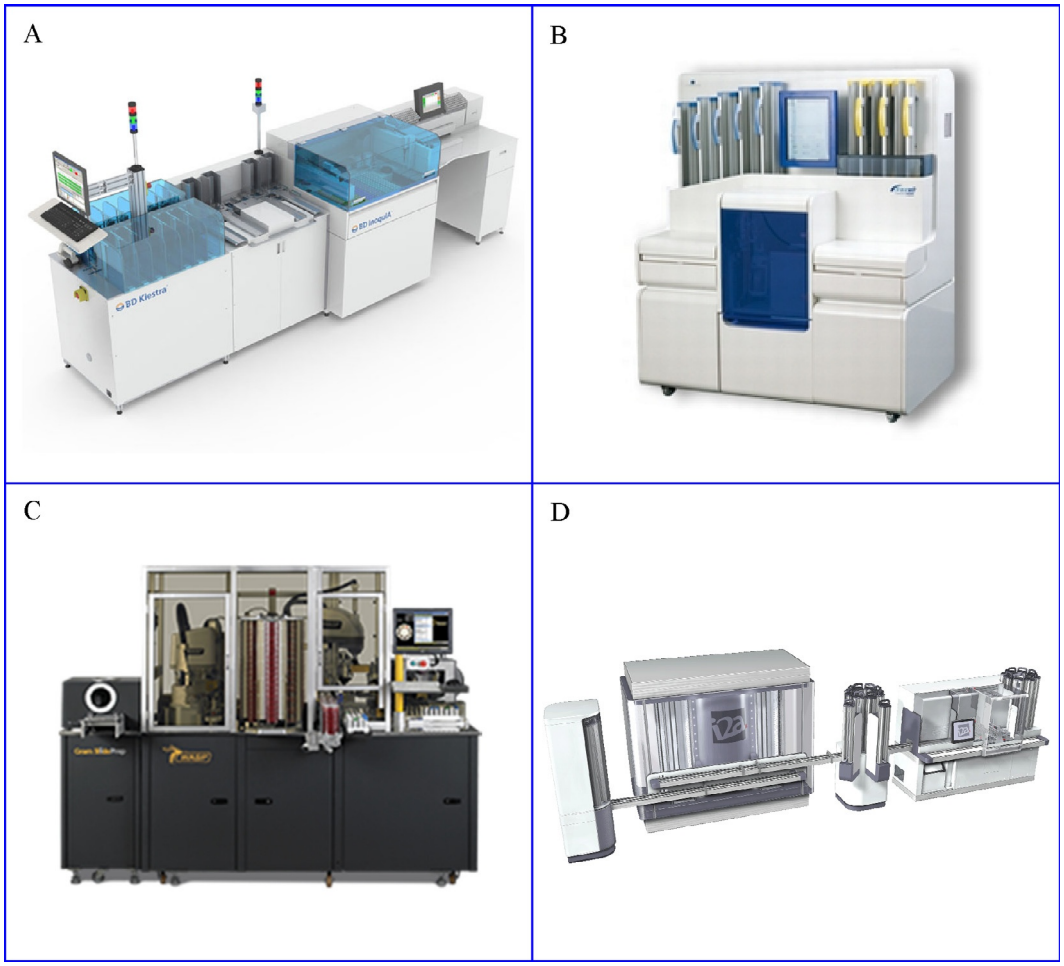


Figure 3 The Beckton Dickenson Kiestra™ InoqulA™ (a) is a fully automated, high throughput machine that processes both liquid and solid samples (photo courtesy of Paul Bourbeau). The InoqulA™ system fully integrates with additional products from the BD Kiestra line which automates incubation, reading, identification, and susceptibility of samples. The bioMieriex Previ Isola (b) provides clinical laboratories with an automated means to quickly and accurately identify and perform antimicrobial susceptibility testing (bioMerieux, 2014). The Walk-Away Specimen Processor (WASP) by Copan/Siemens (c) encompasses all aspects of liquid specimen processing including plate streaking, gram slide preparation, and enrichment broth inoculation (Siemens, 2014). The Sirscan2000automatic by i2a (d) incubates samples and reads samples (photo courtesy of Christian Curel).

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