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Microbiology in Clinical Pathology

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Glossary

Bacteria Single-celled organisms called prokaryotes that lack a nuclear membrane. They are usually 0.5–5.0 μm in length. They may be spherical (cocci) or rod-shaped (bacilli), curved, or spiral-shaped.

Biosafety levels The level of biocontainment precautions required to handle microorganisms. The lowest level, level 1, concerns organisms that are not typically dangerous, while level 4 concerns organisms that are highly dangerous and require many precautions. Most work in a clinical microbiology hospital laboratory is performed under BSL2.

Epidemiology The field of study involving the incidence, distribution, and control of disease. Infection control practitioners and public health authorities track the epidemiology of multiple diseases to contain outbreaks and prevent disease.

Fungi Multicelled eukaryotic organisms including yeasts and molds. Dimorphic molds grow as either yeast or mold, depending on temperature.

MALDI-TOF Matrix-assisted laser desorption ionization time-of-flight mass spectrometry is a method to analyze biomolecules by laser fragmentation and soft ionization within a protective matrix, followed by measurement of spectra of flight times in a vacuum. By analyzing primarily ribosomal proteins, microorganisms are identified to species level in clinical laboratories.

Next-generation sequencing A method to determine the order of nucleotides of DNA using a high-throughput technology that parallelizes the sequencing process, producing many sequences at the same time, utilizing a variety of chemistries for signal detection. These methods are less expensive than older sequencing methods and are used to produce large quantities of precise data.

Parasite An organism that lives within or on another organism. Parasites often have complicated life cycles, utilizing intermediate hosts, and include a wide range of organisms from microscopic protozoa to very large worms.

Quantitative real-time polymerase chain reaction A variant of polymerase chain reaction used to amplify DNA or RNA (if preceded by a reverse transcriptase reaction) by monitoring amplification with a fluorescent signal at each cycle of the reaction and comparing the signal to a standard curve to determine the quantity of starting nucleic acid.

Viruses Submicroscopic infectious agents consisting of RNA or DNA surrounded by a protein coat only capable of replicating inside host cells. Some viruses also have a lipid envelope.

Abbreviations

BSL	Biosafety level
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
DFA	Direct fluorescent antibody
FDA	Food and Drug Administration
FISH	Fluorescence *in situ* hybridization
HIV	Human immunodeficiency virus
MALDI-TOF	Matrix-assisted laser desorption ionization-time of flight
MIC	Minimal inhibitory concentration
MLST	Multilocus sequence typing
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
RFLP	Restriction fragment length polymorphism

Introduction/Overview

Microbes are ubiquitous in nature and number in the thousands. They are both essential for our health and the cause of devastating infectious diseases. Diagnostic microbiology must identify the pathogenic microbes that cause disease and distinguish them from normal flora and from environmental organisms not causing disease. The diagnostic microbiology laboratory is essential for the diagnosis and treatment of infectious diseases. Diagnostic microbiology utilizes methods that have been in place for hundreds of years, such as the Gram stain, and recently developed techniques that allow very sophisticated analysis of gene sequence and protein profiles. Routinely, a hospital laboratory identifies the organisms causing the most common infections, yet laboratory personnel must also be alert for unusual pathogens and for the next epidemic.

Diagnostic microbiology is a subsection of clinical pathology within the hospital laboratory, usually as part of a department of pathology or a department of laboratory medicine. Microbiology laboratories are also located in independent reference laboratories that serve large geographic areas and that offer a broad test menu including more esoteric tests for less common infections. A director of microbiology in a hospital or clinical reference laboratory may take several routes for training, as directors may earn either an MD or a PhD degree. If the director is a physician, then residency in either pathology or infectious diseases is most common, with a possible additional medical microbiology fellowship. If the director has a

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PhD in a biological field as initial training, a 2-year fellowship in medical microbiology is required. Certification may be obtained from the American Board of Pathology or from the American Academy of Microbiology, depending on the training path. Staff working in the hospital laboratory may be trained in a 4-year medical technologist program, or they may receive on-the-job training following an undergraduate 4-year degree in a biological field, or technologist-specific training following the original undergraduate degree. Certification of technologists is completed through the American Society for Clinical Pathology.

The diseases caused by microbes and the diagnostic methods used to detect microbes vary widely based on type of microbe: viruses, bacteria, fungi, or parasites.

Viruses

The very small size of viruses (most are 20–400 nm) and their dependence on host cells for replication drive the methods used to detect them. Viruses typically carry DNA or RNA, not both, surrounded by a protein shell and in some viruses, a lipid coat. Viral infections may be acute or chronic and clinical symptoms of viral infection may be florid or absent. Viruses may cause rapid death of a host cell or integrate viral genetic material into the host genome. Viral infections can be mild and common, such as rhinovirus causing colds, or rare and devastating, such as Ebola virus causing hemorrhagic fever. Viruses may be highly contagious, such as influenza, or have limited transmission capability, such as the human immunodeficiency virus (HIV) (Figure 1).

Bacteria

Bacteria are unicellular organisms lacking a nuclear membrane. The cell wall may have a thick peptidoglycan layer or a thin peptidoglycan layer along with a lipid-containing outer membrane. A few pathogenic bacteria, such as mycoplasma, lack a cell wall. Mycobacteria have a cell wall containing mycolic acid, a property affecting virulence, stability, and our detection methods.

Fungi

Fungi are eukaryotic organisms that exist as a unicellular yeast or a filamentous mold. Yeast replicate asexually, whereas mold may replicate sexually or asexually. A class of fungi called dimorphic fungi have two forms, a yeast form and a mold form. The dimorphic molds *Histoplasma*, *Blastomyces*, and *Coccidioides* are prominent pathogens.

Parasites

Parasites are eukaryotic microbes of vastly diverse forms. Protozoa are quite small, while some parasitic worms are very large. Parasites can have very complex life cycles, evolving through multiple developmental stages in one or two intermediate animal hosts before infecting the definitive host. Parasitic diseases are particularly geography-specific, so knowing the home, travel history, lifestyles, pets, and potential exposures of a patient is a required part of any medical evaluation when creating a parasitic differential diagnosis.

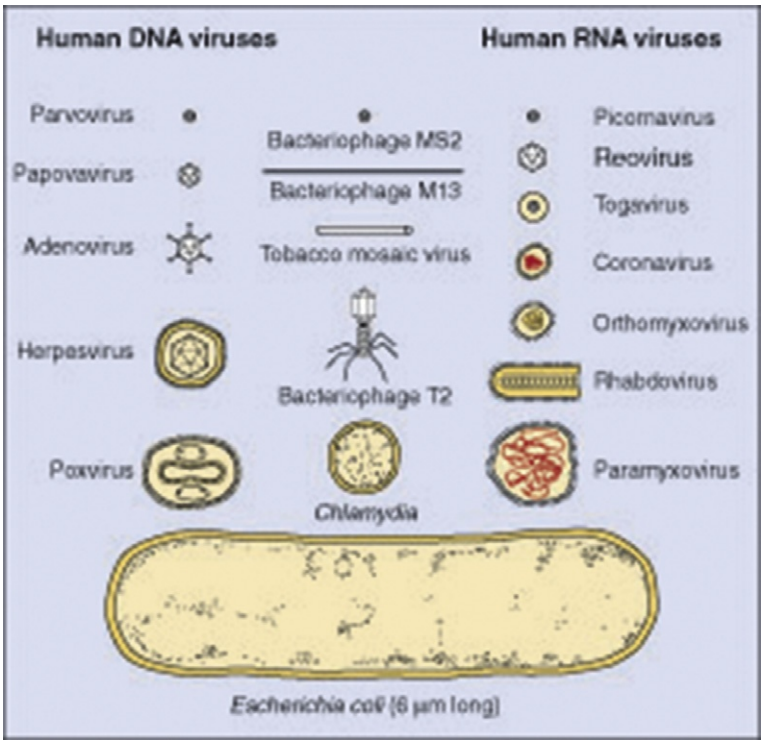

Figure 1 Relative sizes of viruses and bacteria. Reprinted from Figure 44-4 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

Table 1 Examples of normal flora

Organ	*Some representative normal flora*
Upper respiratory tract	*Actinobacillus*, *Actinomyces*, *Candida*, *Cardiobacterium*, *Corynebacterium*, *Eikenella*, *Fusobacterium*, *Haemophilus*, *Kingella*, *Moraxella*, *Mycoplasma*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Propionibacterium*, *Rothia*, *Staphylococcus*, *Streptococcus*
Gastrointestinal tract	*Acinetobacter*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, Enterobacteriaceae, *Enterococcus*, *Fusobacterium*, *Porphyromonas*, *Staphylococcus*, *Blastocystis*, *Chilomastix*, *Endolimax*, *Entamoeba*
Genitourinary tract	*Bacteroides*, *Candida*, *Corynebacterium*, Enterobacteriaceae, *Enterococcus*, *Eubacterium*, *Gardnerella*, *Lactobacillus*, *Mobiluncus*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Propionibacterium*, *Staphylococcus*, *Streptococcus*, *Ureaplasma*
Skin	*Aerococcus*, *Bacillus*, *Candida*, *Corynebacterium*, *Malassezia*, *Micrococcus*, *Peptostreptococcus*, *Propionibacterium*, *Staphylococcus*, *Streptococcus*

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Normal Flora

There are many different microorganisms that colonize humans, in an organ-specific way (Table 1). Normal flora are beneficial to us for their roles in food metabolism, protection against pathogenic microbes, production of growth factors, and balancing the interactions of the innate and adaptive immune system with the environment. It is very important to consider the normal flora when interpreting the results of a positive culture. Some normal flora are very rarely pathogens and would not be treated by physicians. An example would be *Lactobacillus* from a vaginal specimen and most *Corynebacteria* from a wound/skin specimen. Other organisms recovered are always considered pathogens and must be treated. Examples include rabies virus, *Bacillus anthracis*, *Plasmodium*, *Listeria*, or *Mycobacterium tuberculosis*. A third group of organisms are potential pathogens but may colonize some individuals. This group would include viridans streptococci, *Streptococcus pneumoniae*, *Staphylococcus*, and quite a few of the organisms listed in the table of normal flora. Therefore, it is essential that the laboratory results be interpreted in the context of the clinical scenario. Furthermore, there are many environmental microorganisms that are not associated with human disease. The processes routinely used in the clinical laboratory are tailored to detect those organisms known to cause disease.

Biosafety Levels

When analyzing human pathogens, it is essential to understand the risk to the laboratory professional while culturing or processing the samples. There are four biosafety levels (BSLs) of increasing precautions as risk increases. BSL1 involves agents that are not known to cause disease consistently in healthy adults. Most routine work in the hospital laboratory, involving the handling of agents that are associated with human disease, is performed with BSL2 precautions in place. Biosafety cabinets for physical containment devises are used for any steps that cause splashing or aerosolization. Personal protective equipment such as lab coats, gloves, and face protection are used as needed per procedure guidelines. BSL3 levels guide the testing of agents that cause more serious disease and/or have a higher potential for aerosolization such as *M. tuberculosis* and *Coccidioides immitis*. Increased protection is obtained by limited access to the room containing the lab bench, negative airflow into the laboratory, increased use of the biosafety cabinet, and increased steps to decontaminate waste. BSL4 laboratories are much less common and they deal with the most dangerous agents such as hemorrhagic fever viruses. Additional precautions include very restricted access, additional training and monitoring of staff, changes of clothing, and a positive pressure personnel suit.

Clinical Value

Global Burden of Disease

The burden of infectious diseases globally is enormous with approximately a third of deaths globally due to communicable diseases. Although tremendous progress has been made during this past century to reduce morbidity and mortality due to infectious diseases, especially in developed countries, the percentage of deaths due to infectious diseases compared to many other types of human disease is still quite significant. Diseases that have challenged us for centuries, such as tuberculosis and cholera, still infect and kill many people each year. Although there has been a worldwide campaign to eradicate diseases such as polio, this disease remains to be eliminated from the causes of human disease. While long-standing causes of infectious diseases remain prevalent, new emerging infectious diseases add to the challenge of physicians treating patients. Agents including West Nile virus, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus, H5N1 avian influenza virus, HIV, and human metapneumovirus have all appeared or spread widely in recent times. The threat of a pandemic, in which the disease occurs over a very large geographic area, is real, although not a guarantee, and vigilance by public health practitioners and infectious disease specialists around the world will aid in curtailing the next emerging disease. Beyond the confirmed associations of infectious agents with many diseases, there are many cases for which there are limited data to associate a specific disease with an infectious process, and thus, much ongoing research attempts to further determine these associations. More than thirty diseases have some evidence suggesting an infectious agent may be involved, but either no agent has been identified or the evidence is still preliminary. Examples include Crohn's disease, psoriasis, sarcoidosis, diabetes mellitus, chronic fatigue syndrome, and atherosclerosis.

Association of Viruses with Cancer

Surprising to many is the finding that microbes play a role in several malignancies that were previously thought to be

unrelated to infectious diseases. *Helicobacter pylori* has been shown to have a likely role in gastric cancer, and human papillomavirus is a cause of cervical and head and neck cancers. Human herpes virus 8 is a causative agent of Kaposi's sarcoma and Epstein–Barr virus causes lymphoma and possibly Hodgkin's disease. It seems likely, given this change in our understanding of the relationship between cancer and microbes, that additional examples will be discovered.

Role of the Microbiology Laboratory in Accurate Diagnosis

An infectious disease can present with signs and symptoms consistent with multiple noninfectious disease processes, so a positive microbiology result can be the key information to guide therapy. There are multiple dramatic cases of patients who have undergone surgery for treatment of a noninfectious disease or who were being evaluated for malignancies when, unexpectedly, the patients had treatable infectious processes. Effective communication between primary treating physicians, the infectious disease consult team, and microbiology laboratory staff helps to ensure that correct tests are being ordered, with additional stains and media added as needed to cover the differential diagnosis. Furthermore, the correct interpretation of data is required to decide optimal therapy, with test limitations conveyed from laboratory staff to the treatment team. Some typical examples of disease associations with specific bacteria are listed in Table 2.

Table 2 Examples of bacteria–disease associations

Organism	*Disease*
Actinomyces	Cervicofacial, thoracic, abdominal, central nervous system infections
Burkholderia cepacia complex	Pulmonary infections
Campylobacter	Gastroenteritis
Clostridium perfringens	Food poisoning, myonecrosis, soft tissue infections
Eikenella	Human bite wounds
Enterococcus	Urinary tract infections, bacteremia
Enterohemorrhagic *E. coli*	Watery diarrhea, hemolytic uremic syndrome
Haemophilus influenzae	Meningitis, septicemia, cellulitis, respiratory infections, otitis media
Helicobacter pylori	Gastritis, duodenal ulcers, gastric adenocarcinoma
Klebsiella pneumoniae	Pneumonia, urinary tract infections
Legionella pneumophila	Pneumonia
Listeria monocytogenes	Meningitis, bacteremia
Mycoplasma pneumoniae	Atypical pneumonia
Neisseria gonorrhoeae	Gonorrhea, septic arthritis, pelvic inflammatory disease
Pseudomonas aeruginosa	Pulmonary, skin/soft tissue, burns, bacteremia
Salmonella	Diarrhea, enteric fever
Staphylococcus aureus	Suppurative skin infections, bacteremia, osteomyelitis, pneumonia, toxin-mediated infections
Streptococcus pyogenes	Suppurative infections, pharyngitis, scarlet fever, skin and soft tissue infections, toxic shock-like syndrome, rheumatic fever, glomerulonephritis

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Outbreaks

Outbreaks involve many people each year. In 2009–10, the US public health departments reported 1527 foodborne disease outbreaks, resulting in 29 444 cases of illness, 1184 hospitalizations, and 23 deaths. The steps taken to manage a new outbreak include the detection of an outbreak strain in a patient by the clinical microbiology laboratory, followed by investigation of the source of the infection, and virulence, susceptibility, and genetic analysis in public health and research laboratories.

Nosocomial Infections

Nosocomial infections are those acquired in the healthcare system. They are very common and are often associated with the most resistant organisms, making therapy difficult. The infection control team in a healthcare facility monitors nosocomial infections and adherence to isolation guidelines, along with investigation of potential outbreaks or organism transmission within an institution. The diagnostic microbiology laboratory performs testing for surveillance and for transmission investigation. Urinary tract infections are the most common nosocomial infection, followed by surgical wound and respiratory tract infections, and then finally bloodstream infections. New hospital monitoring systems such as the National Healthcare Safety Network now require hospitals to participate in tracking of nosocomial infections. Tracking patients who are colonized with specific resistant organisms, following by implementation of isolation precautions while hospitalized, is of enormous value in protecting susceptible and vulnerable patients from additional infections that could be spread throughout the hospital by healthcare workers. Examples include methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, vancomycin-resistant *Enterococcus*, respiratory viruses, and multidrug-resistant Gram-negative enterobacteriaceae such as *Klebsiella pneumoniae* that are positive for the carbapenemase enzyme, bla_{KPC}.

Legislative Considerations

Some infections have become a major concern for public health agencies and to the general public. Given the spread of MRSA in recent years, some state governments in the United States have established legislation requiring specific types of monitoring in hospitals, such as in the intensive care units. Implementation of this legislation was very controversial, as many physicians felt that although important to monitor, action would be best decided by a hospital's infection control team based on data in that hospital, instead of being legislated by the regional government without the flexibility of adjusting quickly to changing needs for monitoring infections as trends

change. Flexibility would permit assigning resources to the most essential areas of concern at any one time. Many countries have guidelines established by consensus committees of infectious disease organizations to control the spread of resistant organisms, even if there is no specific legislation regarding this issue.

Susceptibility Testing

Susceptibility testing is one of the most critical functions of the microbiology laboratory. It is best to use the minimal number of drugs that will effectively treat an infection and physicians often start broad-spectrum drugs as empirical therapy until further laboratory data are available. If the broad-spectrum antimicrobial agent is not replaced with the optimal drugs, toxicity may be worse, and spread of resistance throughout the world is a very significant consequence of widespread use of antibiotics.

Multiple drugs to treat bacteria, fungi, viruses, and parasites have been developed since the 1940s; however, microbes have evolved to counter the effectiveness of these agents in many cases. Resistance has developed and spread due to mutations in genomes or plasmids, small circular DNA strands that replicate independently of the chromosome, combined with the transfer of resistance genes from one species to another. Some strains of resistant bacteria are very common, such as methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and penicillin-resistant *S. pneumoniae*. Some multidrug-resistant organisms are resistant to all available antimicrobial agents in our arsenal, which is devastating for the patients who get these infections.

By identifying an infectious agent as the cause of a set of symptoms, a patient may be spared further unnecessary invasive procedures to diagnose his condition. Clearly, the accurate diagnosis of the responsible infectious agent is of extreme importance to properly treat individual patients, to follow and stop the spread of disease within the population, and to increase our scientific understanding of the pathogenesis of both old and new infectious diseases. Further, staying up-to-date on improvements in our diagnostic assays and advances in technology is a key part of the job of a medical microbiologist.

General Principles of Methods

Specimen Collection

Proper specimen collection is essential for accurate diagnosis of infectious diseases. The correct specimen type must be sent in the correct transport container at the appropriate temperature, without unnecessary delay. These are important issues that are constantly monitored by hospital laboratory services. Examples of common problems include a poorly collected urine specimen that is contaminated by skin/vaginal/fecal flora, a viral culture that is not collected in viral transport medium containing antibiotics to kill bacteria so that the culture is overgrown by bacteria, or failure to collect infected wound tissue in anaerobic transport media even though anaerobes are likely pathogens in the case. Some RNA viruses are labile and culture positivity would decline with a delay in transport. Fortunately, most viral testing is now molecular-based testing, removing the requirement for viable organism for detection, so this issue is not as prevalent at this time. Collection of the appropriate amount of blood for blood cultures in potentially septic patients is critical for optimal detection of the causative microorganism. To improve testing effectiveness, accrediting organizations, such as the College of American Pathologists, have recently added requirements for ongoing monitoring and feedback to clinicians on the blood volumes collected from patients. An improperly collected specimen may result in a missed diagnosis, or alternatively, the report and subsequent treatment of a patient, for an organism that is a commensal or contaminant, along with discontinuation of testing before the true cause of infection is identified. On the appearance of new symptoms in a patient, collection of a specimen prior to antimicrobial administration is important to optimize the potential for a positive culture. This is especially true in a septic patient diagnosed using blood cultures. If the specimen volume is small and many tests are requested, the physician must prioritize the testing, as the limited volume may not be adequate for the initial requested set of assays. Most processing and plating of specimens for culture is done manually by technologists; however, automated systems have been developed to handle and plate specimens. Three examples of automated systems include Kiestra TLA (BD Kiestra B.V., Drachten, the Netherlands), Full Microbiology Laboratory Automation (FMLA; bioMérieux, Inc., La Balme, France), and the WASPLab (Copan Diagnostics, Murrieta, CA). The high cost of such a system and the inability of the systems to handle unusual containers, small volumes, and complicated test panels result in the systems being most appropriate for large reference laboratories or high-volume laboratories.

Stains and Microscopy

Microscopy remains critical to microbiology workflow, with light microscopy and fluorescent microscopy being used most frequently. Direct smears of patient specimens permit detection of infection without any antecedent culture, a rapid route to a preliminary or definitive diagnosis. Bacteria are divided into Gram-positive and Gram-negative organisms based on a historic stain using crystal violet and a safranin counterstain, with distinguishes based on cell wall peptidoglycan thickness and membrane lipid content (Table 3). The Gram stain is the most frequently used stain in the microbiology laboratory (Figures 2–5). Mycobacteria can be detected with a Kinyoun acid-fast stain or a fluorescent stain (Figure 6). A calcofluor white stain or Fungi-Fluor fluorescent stain of direct specimens is frequently used to detect fungal elements. Examples of fungal stains are shown in Figures 7–13. Multiple fluorescent stains are utilized for specific identification of viral and parasitic organisms incorporating a monoclonal antibody for specificity. Iodine preparations, acid-fast stains, and trichrome stains are useful for parasitic identifications. Examples of stains for parasites are shown in Figures 14–20. The 100× oil objective of the microscope is most commonly used for bacterial microscopy, and 40× dry objective is often used for wet mounts and fluorescent stains. Dark field microscopy, which uses an illumination technique to enhance contrast in unstained specimens producing bright objects on a dark background, can be used to detect spirochetes and *Leptospira*, but is

Table 3 Commonly used stains

Stain	*Principle and application*
Acridine orange	Stains DNA of bacteria and fungi
Auramine–rhodamine	Fluorescent stain for mycobacteria
Calcofluor white or Fungi-Fluor fluorescent	Used to detect fungi
Gram	Bacteria are divided into Gram-positive and Gram-negative organisms based on cell wall peptidoglycan thickness and membrane lipid content
Kinyoun	Acid-fast stain for mycobacteria
Lugol's iodine	Wet preparation of parasitology specimens
Methenamine silver	Stains fungal elements in tissue
Modified acid-fast	Stain for *Nocardia*, *Rhodococcus*, *Tsukamurella*, *Gordonia*, *Cryptosporidium*, *Isospora*, *Sarcocystis*, and *Cyclospora*
Trichrome	Stain for protozoa
Wright–Giemsa	To detect blood parasites, viral and chlamydial inclusion bodies, *Borrelia*, *Toxoplasma*, *Pneumocystis*, and *Rickettsia*
Ziehl–Neelsen S	Acid-fast stain for mycobacteria

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not routinely used. Phase contrast microscopy converts phase shifts in the light passing through the transparent specimen into brightness changes that are visible as cellular structures and is used for examining unstained material in wet mounts and cell cultures.

Fungi

In most laboratories, identification of yeast can in many cases be accomplished with instruments that identify bacteria, but identification of mold involves microscopic analysis of slide culture, looking for hyphal elements and specialized hyphal structures, called conidiophores that produce fungal spores called conidia. This requires time and expertise. Some labs have begun to use mass spectrometry for identification of fungi, greatly reducing the time and increasing the accuracy of identification.

Parasites

The burden of disease due to parasites varies tremendously based on geographic location, with a much higher burden in developing countries. Parasitology is the area of the laboratory that still uses the most traditional methods of multiple stains and direct microscopic examination. Several multiplex molecular panels have been developed that include organisms such as *Giardia* and *Entamoeba*, in addition to pathogenic bacteria

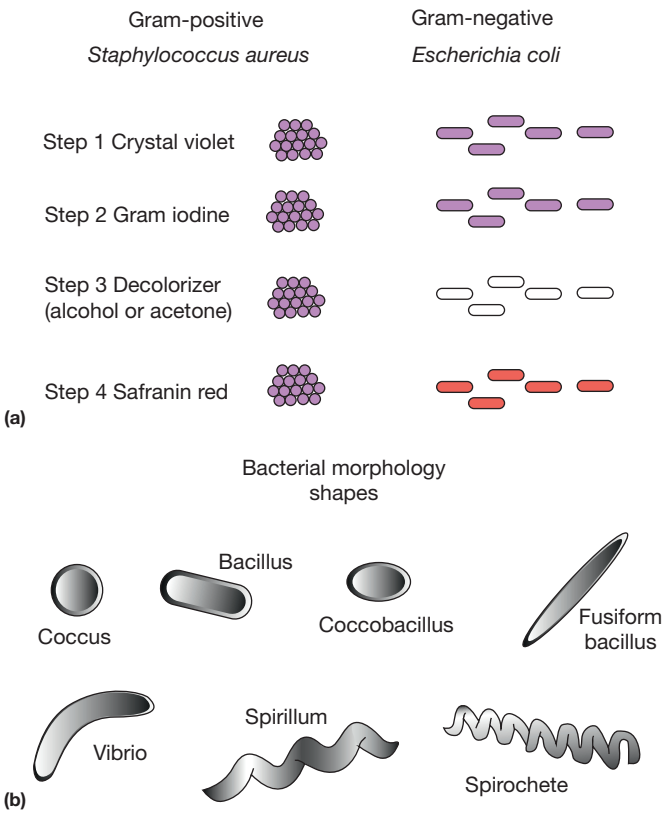

Figure 2 Gram stain morphology of bacteria. (a) Crystal violet of Gram stain is precipitated by Gram iodine and is trapped in the thick peptidoglycan layer in Gram-positive bacteria. The decolorizer disperses the Gram-negative outer membrane and washes the crystal violet from the thick layer of peptidoglycan. Gram-negative bacteria are visualized by the red counterstain. (b) Bacterial morphologies. Reprinted from Figure 12-3 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

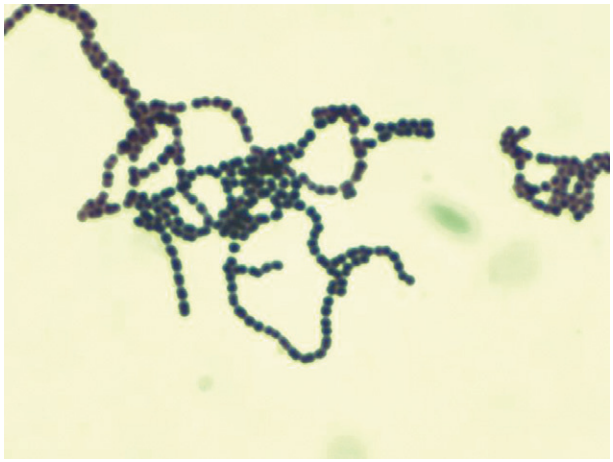

Figure 3 Gram stain of *Streptococcus pyogenes*. Reprinted from Figure 19-1 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

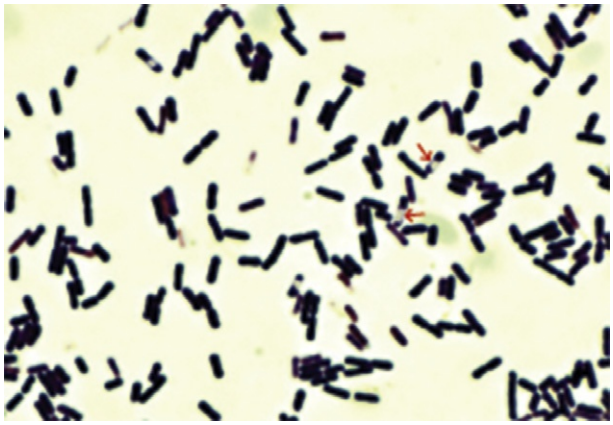

Figure 4 Gram stain of *Bacillus cereus*. The clear areas in the Gram-positive rods are unstained spores (arrows). Reprinted from Figure 21-1 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

and viruses, but currently, most routine labs have not incorporated molecular methods for parasites into the daily workflow. Stool samples are examined by iodine-stained wet mount and permanent stains of fixed specimens. Motile protozoa may be seen on wet mount, and eggs and larva may be seen on permanent smear. A tremendous amount of information and definitive identification of pathogens can be made from direct examination of stool (Figure 21).

Specimen quality control through microscopy

Microscopy can be critical for determining appropriateness of a specimen for testing. The examination of a sputum sample for the presence of neutrophils and for relatively few squamous epithelial cells indicates an appropriate specimen for evaluation of a potential pneumonia, whereas a sample with many squamous epithelial cells indicates that it is contaminated with oral cells and flora and is thus a poor specimen. Patients who are immunocompromised may not produce neutrophils, so the criteria for appropriate specimens must be adjusted for these patients. As testing has shifted considerably to molecular methods without a microscopic examination of the specimen, the quality control measure has now been removed from many cases.

Culture Methods

Bacteria and fungi

Culture techniques are still essential for infectious diseases identifications, even with the huge increase in molecular testing, because pathogenic organisms that cause disease must be separated from other organisms in mixed cultures, and the prevalence of one organism compared to other organisms in the culture is useful for assessing the likelihood that an organism is contributing to disease. Molecular techniques cannot substitute for this information at this time. Enriched nonselective media that allow growth of many organisms include blood agar, chocolate agar, and thioglycollate broth (Table 4). Selective media promote the growth of one group of organisms while inhibiting others. Differential media permit the distinction of different organisms growing on the same media. Selective and differential media include MacConkey agar for Gram-negative bacteria, which permits the distinction of lactose-fermenting organisms due to a pink color of the colonies. Specialized media will promote the growth of a specific organism while inhibiting most other organisms, such as cefsulodin–irgasan–novobiocin agar for *Yersinia*. To obtain isolated colonies for identification, the agar plates must be streaked appropriately (Figure 22). To culture bacteria, the appropriate temperature and gas mixture is essential. Most bacterial are cultured at 35–37 °C in 5% CO_2. Organisms may grow in an aerobic (oxygen rich) environment or in an anaerobic (lacking oxygen) environment, or they may be microaerophilic, requiring a low concentration of oxygen, or capnophilic, requiring higher CO_2 concentrations for optimal growth. Fungal culture medium contains antibacterial compounds to inhibit bacterial growth and plates are cultured at 30 °C. Mycobacteria cultures require specialized media and long incubation times, up to weeks compared with the short incubation times of <24 h for many bacterial cultures. Examples of various colony morphologies on different types of media are shown in Figures 23–27.

Blood cultures

Automated instruments are used to monitor blood inoculated into broth for blood cultures. The BACTEC system (Becton Dickinson, Sparks, MD) measures the CO_2 produced by the growing organism. The fluorescent signal in the vial is increased in response to increasing CO_2. Continuous monitoring of the signal and the presence of an alarm when a bottle is positive results in a much more rapid detection of sepsis than in days prior to such instruments. The BacT/ALERT (bioMérieux, Durham, NC) has a sensor response to changes in CO_2 resulting in a color change detected by the instrument.

Viral culture methods

Viruses are either DNA or RNA, enclosed in a protein coat with or without a lipid membrane shell. Viruses require host cells

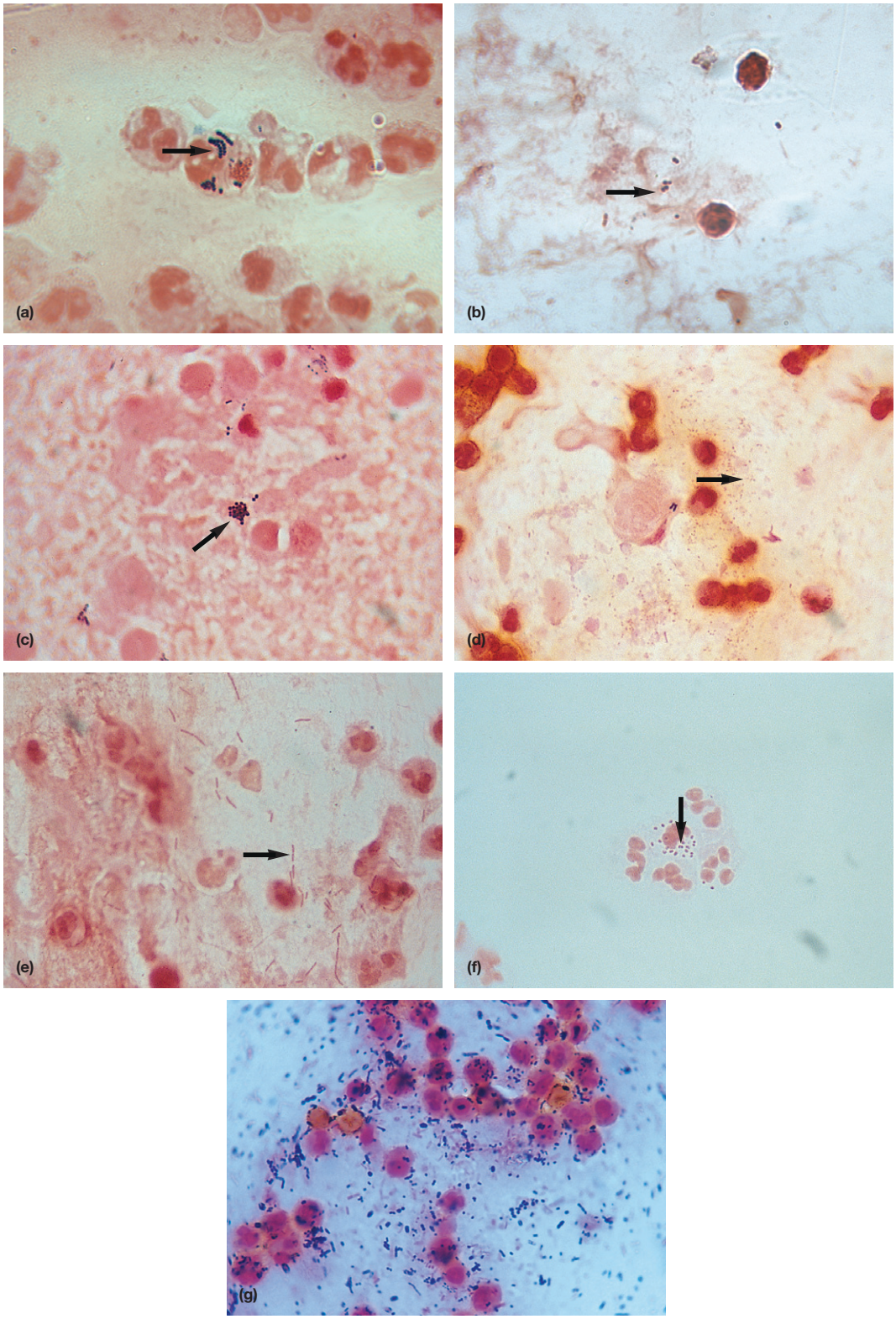

Figure 5 Gram stain direct smears showing polymorphonuclear leukocytes, proteinaceous debris, and bacterial morphologies (arrows), including Gram-positive cocci in chains (a), Gram-positive diplococci (b), Gram-positive cocci in clusters (c), Gram-negative coccobacilli (d), Gram-negative bacilli (e), Gram-negative diplococci (f), and mixed Gram-positive and Gram-negative morphologies (g). Reprinted from Figure 6-6 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

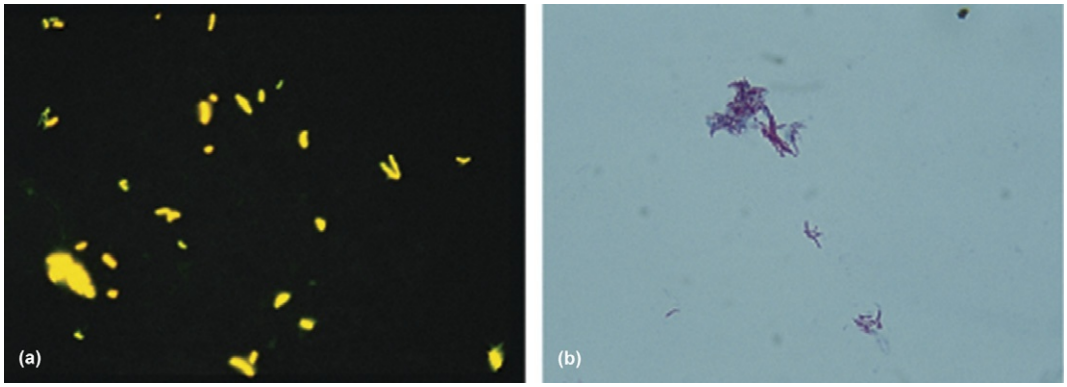

Figure 6 *Mycobacteria tuberculosis* stained with (a) fluorochrome stain (400 × magnification) and (b) Kinyoun acid-fast stain (1000 × magnification). Reprinted from Figure 45-2 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

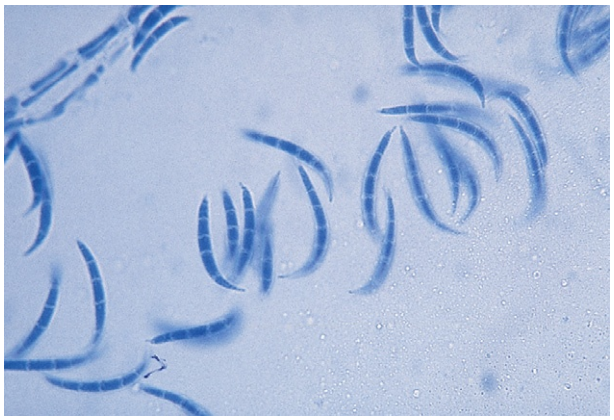

Figure 7 *Fusarium* species showing characteristic multicelled sickle-shaped macroconidia (500 ×). Reprinted from Figure 50-62 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

for replication and many have limited survival outside the host. Traditional detection of viral infections involves culturing the viruses with mammalian cells. Viruses often display cell tropism (preferential growth) for specific cell types and produce characteristic cytopathic (cellular disruption) effects (Figure 28). An adaptation of viral culture is the shell-vial culture during which the specimen is centrifuged into the cells growing on a coverslip. After 2–5 days of incubation, the coverslip is stained with a fluorescently labeled monoclonal antibody specific for the virus in question. Although viral culture methods have disappeared from many routine laboratories, the viral culture techniques are essential for public health and epidemiological data collection, vaccine development, research, and response to emerging viruses. Several pathogens are difficult to culture or noncultivatable in a clinical laboratory, including *Treponema*, *Rickettsia*, *Ehrlichia*, many parasites, Epstein–Barr virus, norovirus, and human papillomaviruses, among others. Alternative methods, such as serology and molecular techniques, must be used to identify these infections. Some examples of virus–disease associations are listed in Figure 29.

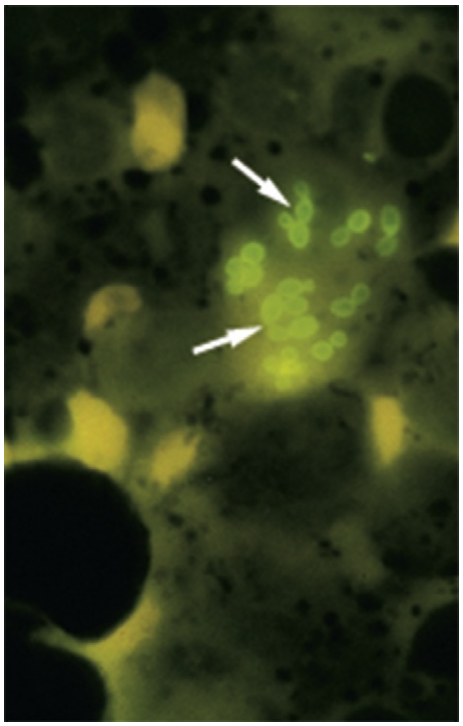

Figure 8 Calcofluor white stain of sputum showing intracellular yeast cells (arrows) of *Histoplasma capsulatum*, which are 2–5 μm in diameter. Reprinted from Figure 50-16 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

Identification Methods

Morphology

Organisms are identified phenotypically based on observable or detectable characteristics, such as morphology, staining properties, growth properties, nutritional requirements, antigenic properties, and resistance profiles. The colony morphology on a plate provides a lot of information for the experienced microbiologist. Some colony characteristics to consider include color, size, shape, whether a colony is convex or concave, edge uniformity, wrinkling of colonies, pitting of agar,

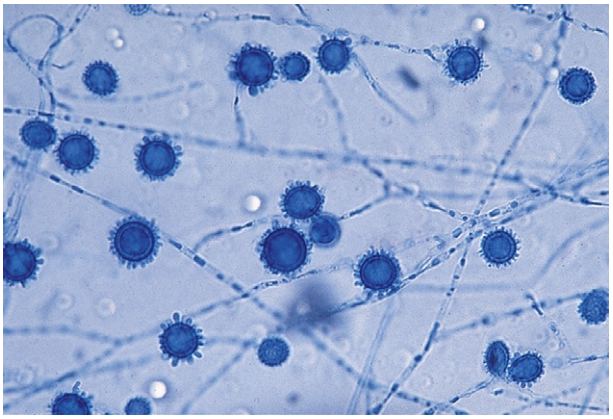

Figure 9 The mycelial form of *Histoplasma capsulatum* produces characteristic tuberculate macroconidia (1000 ×). Reprinted from Figure 50-74 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

Figure 10 *Bipolaris* species showing dematiaceous multicelled conidia produced sympodially from geniculate conidiophores (430 ×). Reprinted from Figure 50-84 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

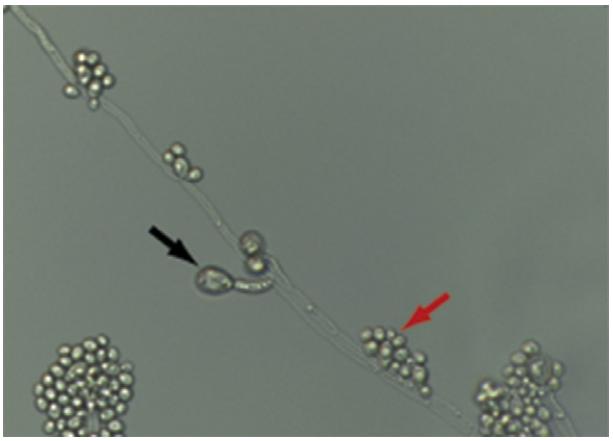

Figure 11 *Candida albicans*, microscopic morphology in cornmeal agar showing large chlamydospores (black arrow), blastoconidia (red arrow), hyphae, and pseudohyphae. Reprinted from Figure 73-2 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

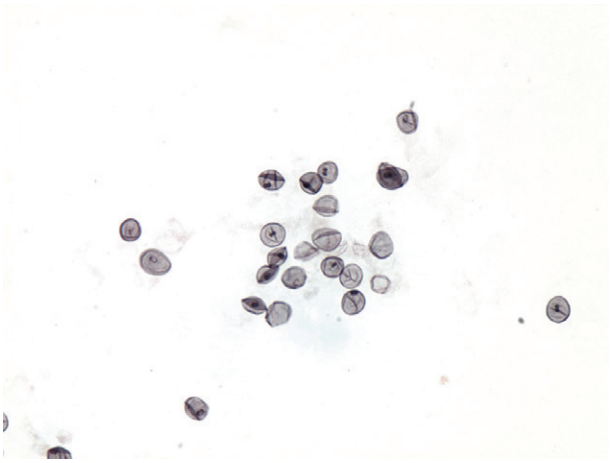

Figure 12 Silver stain of *Pneumocystis jirovecii* cysts. Reprinted from Figure 68-5 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

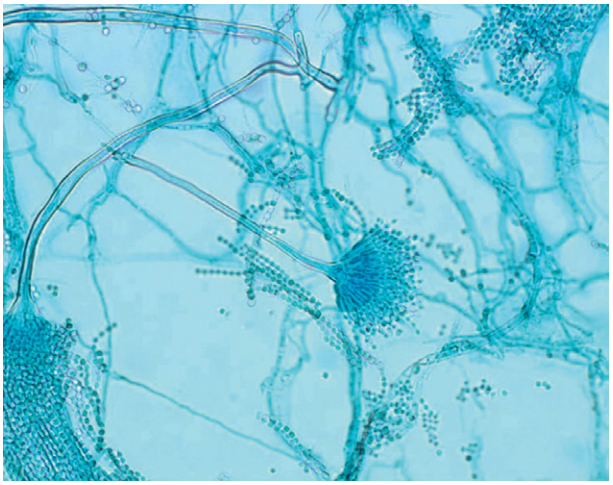

Figure 13 *Aspergillus terreus*. Lactophenol cotton blue preparation showing conidial head. Reprinted from Figure 73-13 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

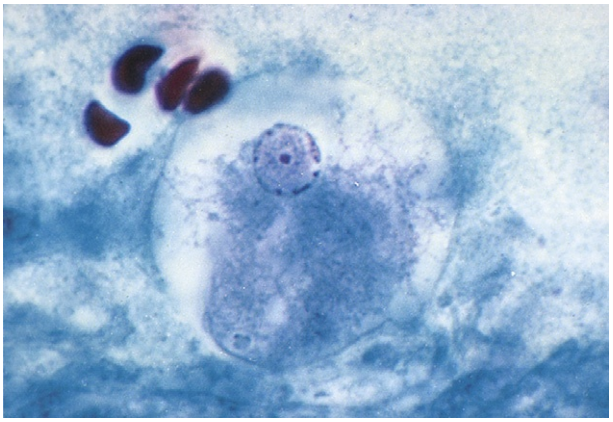

Figure 14 *Entamoeba histolytica*/*E. dispar* trophozoite; no ingested red blood cells are present. Reprinted from Figure 49-15 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

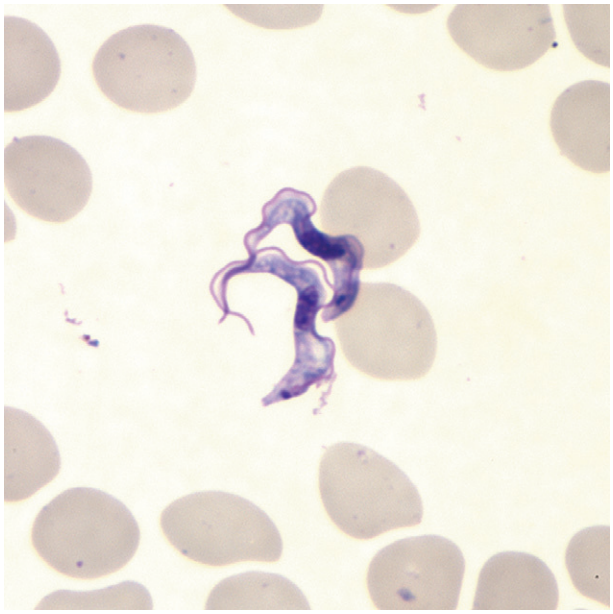

Figure 15 Trypomastigote stage of *Trypanosoma brucei gambiense* in a blood smear. (From CDC Public Health Image Library.)

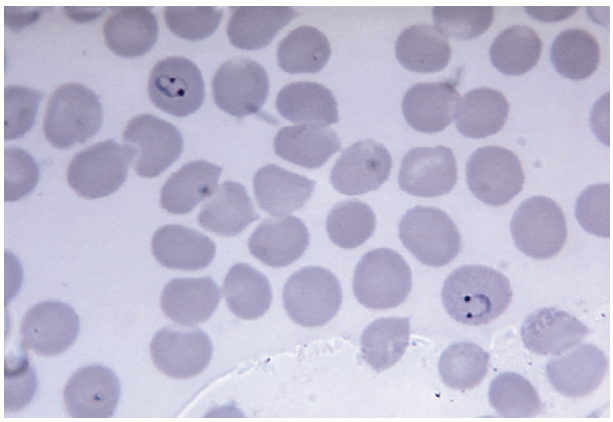

Figure 16 *Plasmodium vivax* ring forms with double chromatin dots. (From CDC Public Health Image Library.)

and hemolysis on a blood plate. Colonies may be glistening and mucoid or very dry, opaque, or transparent. Examples include strong hemolysis for *Streptococcus pyogenes*, pitting of agar by *Eikenella*, and wrinkling of colonies by *Pseudomonas stutzeri*. Specific organisms may also have characteristic smells due to chemicals released by the organisms. *Eikenella* smells like bleach, *Streptococci* can smell like butter, and *Pseudomonas aeruginosa* smells like grapes.

Rapid biochemical reactions

Microbiology laboratories still perform a number of rapid biochemical reactions on bacterial isolates to narrow differential diagnosis quickly. Examples include tests for oxidase, L-pyrroglutamyl aminopeptidase, and catalase, and the *S. aureus* latex bead test for protein A and clumping factor. An enzyme or identifying molecule in the bacteria can be detected in minutes with the use of a substrate that provides a visible change (color

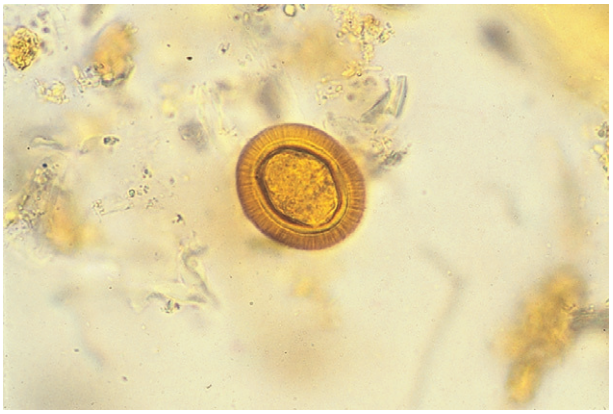

Figure 17 *Taenia* egg. The eggs are spherical, 30–40 μm in diameter, and contain three pairs of hooklets internally. Reprinted from Figure 85-4 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

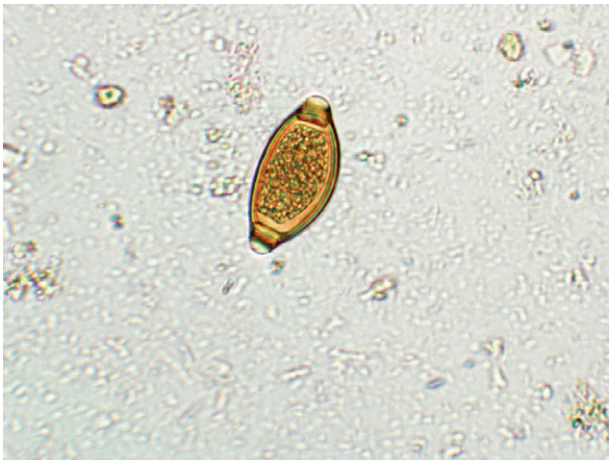

Figure 18 *Trichuris trichiuria* egg. The eggs are barrel-shaped, measuring 50 × 24 μm, with a thick wall and two prominent plugs at the ends. Internally, an unsegmented ovum is present. Reprinted from Figure 83-7 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

change, bubbling, and agglutination) in the presence of the specific bacteria. An example is shown in Figure 30.

Phenotypic identification

Historically, analysis of metabolic activity through detection of substrate utilization involved many biochemical tubes per isolate to determine a phenotypic profile or signature of each bacterial isolate. This profile was compared to a database to match the unknown to a unique species profile. Later, commercial systems were developed to miniaturize and automate the analysis of phenotypic properties. The VITEK 2 (bioMérieux), Phoenix (Becton Dickinson), MicroScan WalkAway (Dade Behring), and Sensititre (TREK/Thermo Scientific) are examples of commercial systems. An automated system may include a filling unit to inoculate a bacterial solution into many wells of a cartridge, an incubator, a reader to detect

changes in substrate or pH indicator following a reaction, and a computer module to make the initial interpretation of the data. One cartridge may contain about 40–45 tests for one organism. For some organisms with typical profiles, the report can be transmitted directly into the medical record for the treating physician to see without the intervention of a technologist in the laboratory. Results can be available in 2–18 h after inoculation. The software is built so that any profile that does not match well will be blocked from transmission to the physician until further investigation is completed by the laboratory, with likely additional follow-up testing.

Mass spectrometry

The introduction of mass spectrometry into routine clinical microbiology laboratories has revolutionized the field. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry is a rapid and reliable identification system with low reagent costs and low labor time after initial investment in an expensive instrument and software. Isolated colonies can be placed directly onto a slide with a matrix solution that absorbed some of the energy of the laser targeted to the bacteria. The laser beam disrupts the bacteria, releasing molecules of various sizes into a vacuum tube toward a detector. Molecules travel based on size and charge, with ribosomal proteins being a predominant component of the analysis. The resulting spectrum for each unknown patient isolate is compared to the profile for many bacteria in a database and matching of data provides the identification of genus or species. An updated and comprehensive database is critical for optimal results. An identification that previously required 18 h to several days can now be completed in 10 min (Figures 31 and 32).

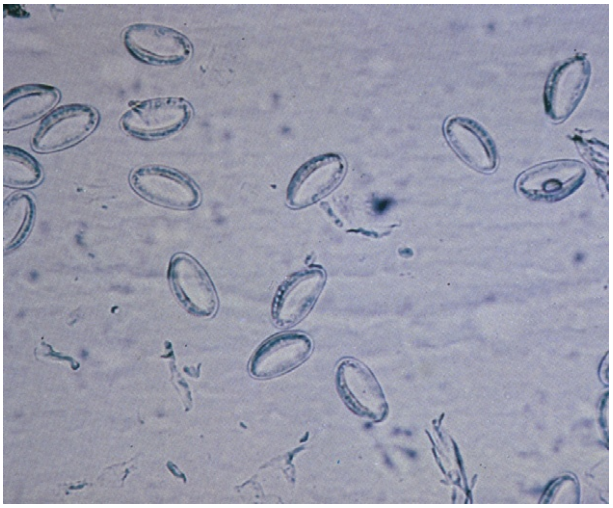

Figure 19 *Enterobius vermicularis* eggs (cellophane (Scotch) tape preparation). Reprinted from Figure 49-69 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

Rapid immunoassays

Lateral flow immunochromatographic assays are very rapid and easy to perform (Figure 33). They can be performed with minimal training and provide rapid results. The assays can identify bacterial antigens in urine, such as for *S. pneumoniae* and *Legionella pneumophila*. These methods detect an infection more rapidly than methods that require culture incubation time. Rapid antigen assays are also used for respiratory viruses, such as influenza and respiratory syncytial virus. The review of literature by the Centers for Disease Control and Prevention (CDC) has shown low sensitivity of some of these tests, and although they are popular in some emergency and primary care settings, rapid immunoassays for respiratory viruses should be used with caution, if at all. A different format of immunoassay for HIV has been used widely to obtain rapid diagnosis of HIV infection in multiple clinical settings.

Mycobacterial identification

Testing for tuberculosis is performed in BSL2 facilities with BSL3 practices. Mycobacteria have a cell wall that includes mycolic acid, giving them different staining properties. This

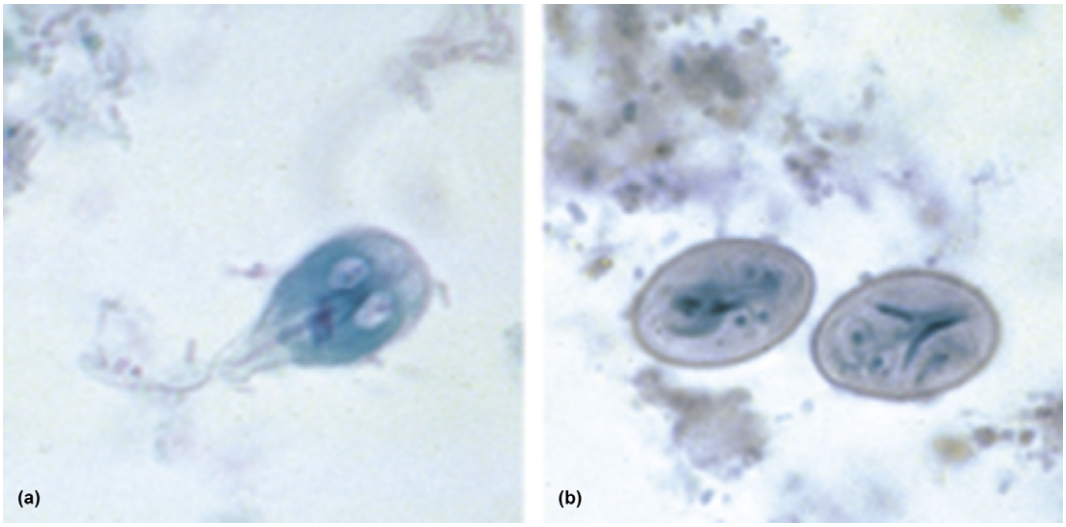

Figure 20 *Giardia lamblia* trophozoite (left). *G. lamblia* cyst (right). Reprinted from Figure 49-31 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

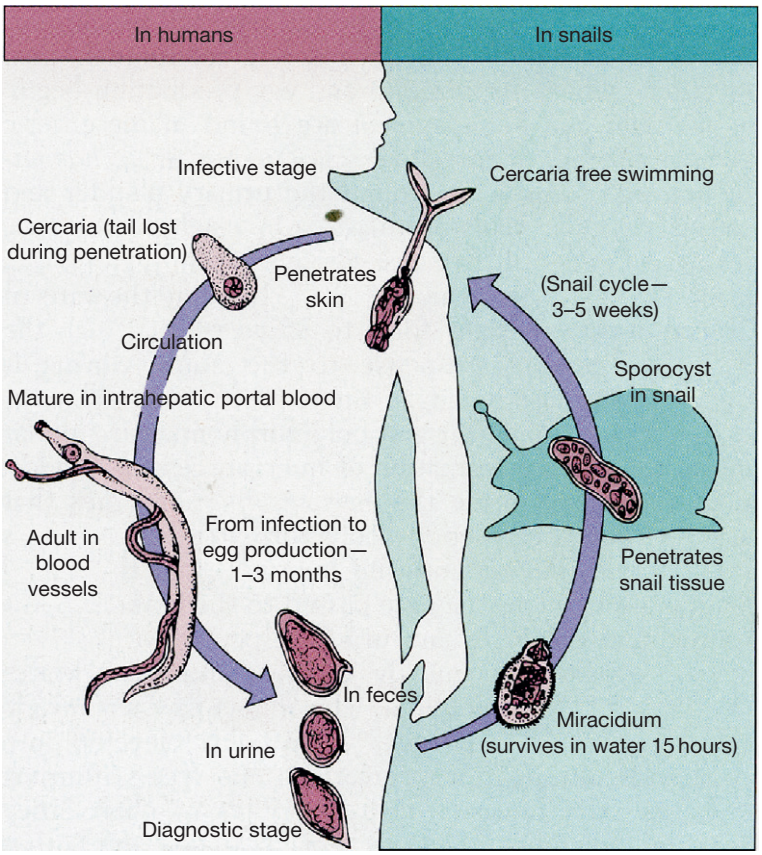

Figure 21 Example of a parasite life cycle: *Schistosoma*. Reprinted from Figure 84-9 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

Table 4 Commonly used media

Media	*Use*
Anaerobic colistin–nalidixic acid agar	Selective for anaerobic *Streptococci*
Bacteroides bile esculin (BBE) agar	Selective and presumptive identification of *Bacteroides fragilis* group
Brain heart infusion media	Permits growth of fastidious organisms
Brucella agar	Permits growth of fastidious organisms including *Brucella*
Buffered charcoal–yeast extract agar	Selective for *Legionella*
Burkholderia cepacia selective agar	Selective for *Burkholderia cepacia*
CDC anaerobe 5% sheep blood agar	For fastidious and slowly growing obligate anaerobes
Cefsulodin–irgasan–novobiocin (CIN) agar	Selective and differential for *Yersinia enterocolitica*
Chocolate agar	For fastidious organisms
Chopped meat broth	For anaerobes
Columbia blood agar	Appropriate for multiple aerobes
Columbia colistin–nalidixic acid	Selective for Gram-positive organisms
Egg yolk agar	Differential for *Clostridium* species
EMB agar	Selective and differential for Gram-negative enteric organisms
Hektoen enteric agar	Selective and differential for Gram-negative enteric organisms
MacConkey agar	Selective and differential for Gram-negative enteric organisms
Middlebrook 7H9 agar	For isolation of mycobacteria
Mueller–Hinton agar	For antimicrobial susceptibility testing
Regan–Lowe media	For *Bordetella*
Thayer–Martin media	For fastidious organisms, especially *Neisseria*
Tryptic soy blood agar	Appropriate for multiple aerobes

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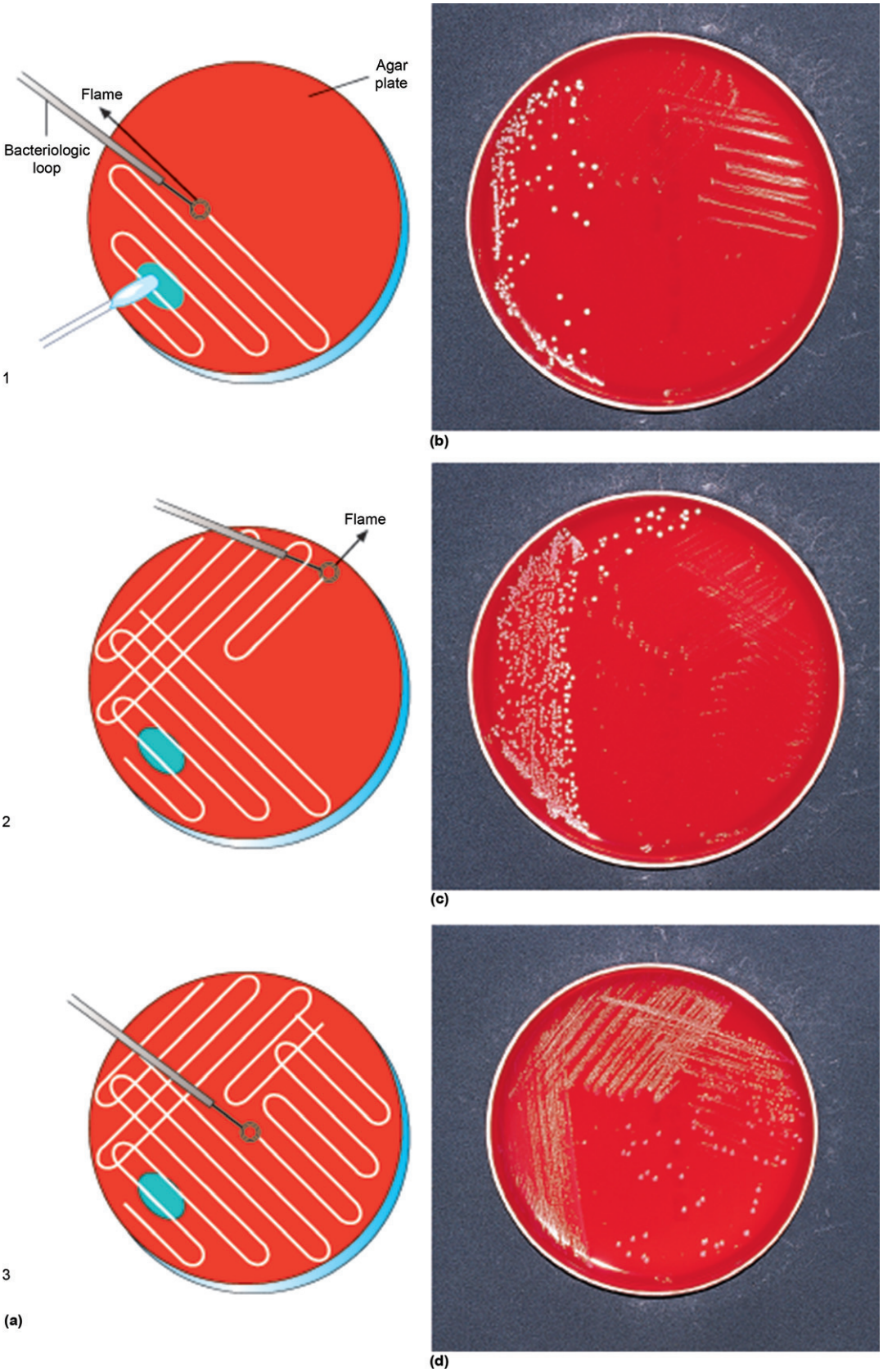


Figure 22 (a) Dilution streak technique for isolation and semiquantification of bacterial colonies. (b) Actual plates show sparse, bacterial growth that is limited to the first quadrant. (c) Moderate bacterial growth that extends to the second quadrant. (d) Heavy bacterial growth that extends to the fourth quadrant. Reprinted from Figure 7-9 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

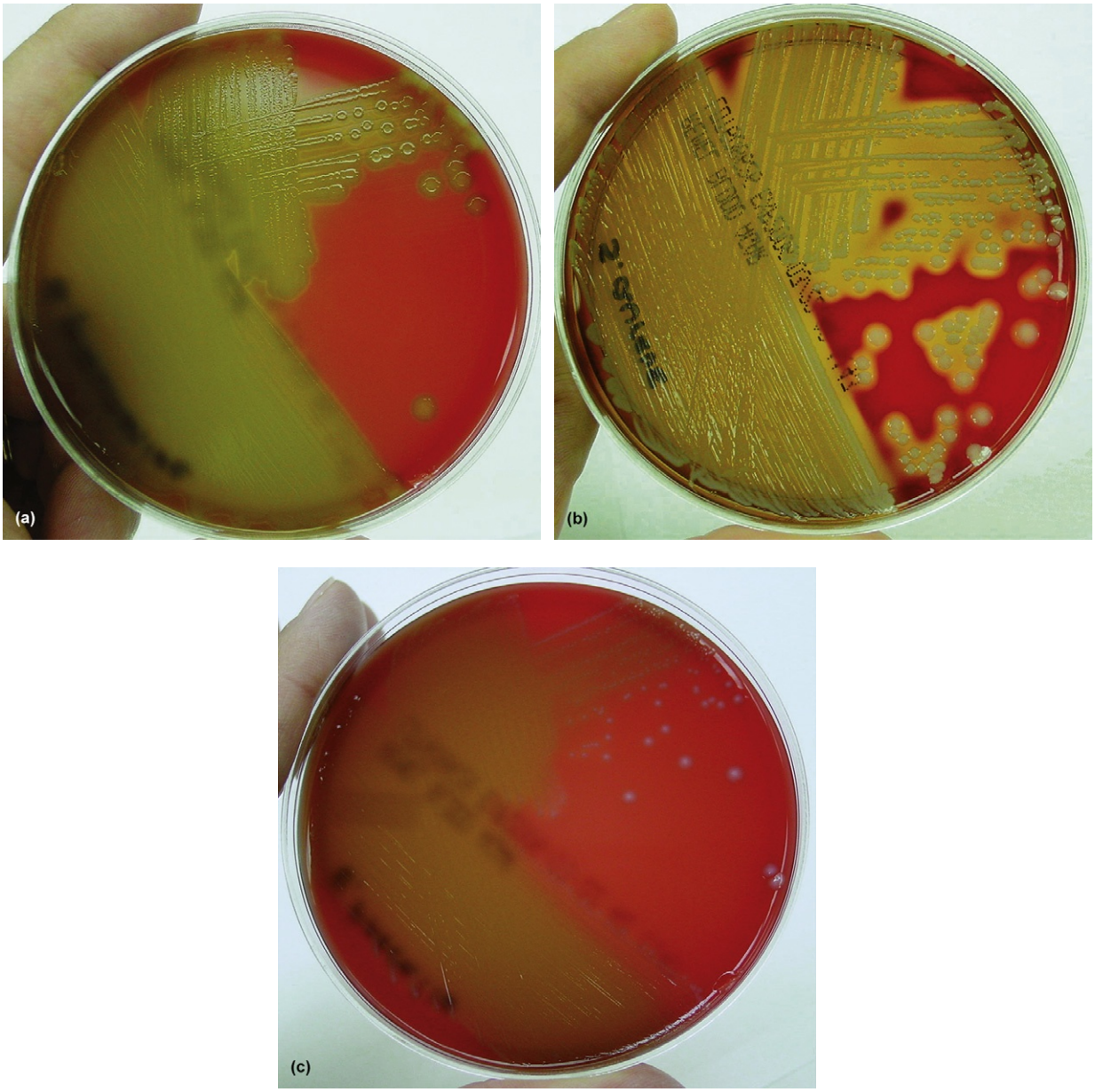


Figure 23 Examples of various types of hemolysis on blood agar. (a) *Streptococcus pneumoniae* showing alpha hemolysis (green around colony). (b) *Staphylococcus aureus* showing beta hemolysis (clear around colonies). (c) *Enterococcus faecalis* showing gamma hemolysis (no hemolysis). Reprinted from Figure 5-2 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

feature is utilized in the acid-fast bacilli stains such as Kinyoun and auramine. Both solid and liquid media for mycobacteria are tailored to the nutrient requirements of these organisms and inhibit the growth of other organisms. Rapidly growing mycobacteria may be detected on routine bacterial media. Nontuberculosis mycobacteria are being reported as a cause of human disease much more now than previously. Broth-based culture systems with automated monitoring exist for mycobacteria, analogous to the blood culture systems. These dramatically improve turnaround time for growth of mycobacteria, as some species can take weeks to grow on solid media. Polymerase chain reaction (PCR) on direct specimens, extraction of mycobacterial isolate DNA for PCR followed by sequencing, use of molecular probes, and MALDI-TOF are all methods in use today to identify mycobacteria as alternatives to the much slower conventional biochemical tests.

Fungi

Yeast are identified by morphological characteristics and assimilation and/or fermentation of carbohydrates. Some yeast are now identified by the multiple types of automated systems used for bacteria, with a final confirmation of identification requiring that the colony characteristics match the organism reported. To identify molds, traditionally morphological study of asexual reproductive characteristics is of key importance. Both yeast and molds can now be identified by

MALDI-TOF, which is extremely rapid compared to traditional mold identification techniques.

Direct fluorescent antibody assays

Direct fluorescent antibody (DFA) assays are used to diagnose varicella zoster virus in skin lesions or *Toxoplasma gondii* in respiratory specimens. A monoclonal antibody directed against a unique antigen on the organism is conjugated to a fluorescent marker that can be seen with a fluorescent microscope. For positive identification, the morphology of the organism or cells must be distinguishable from background artifactual fluorescence. PCR assays are now available and often more sensitive than DFA. The PCR assays are used instead of traditional methods or in combination when the DFA can be performed more quickly or when the information is still felt to provide evidence of the magnitude of the infection.

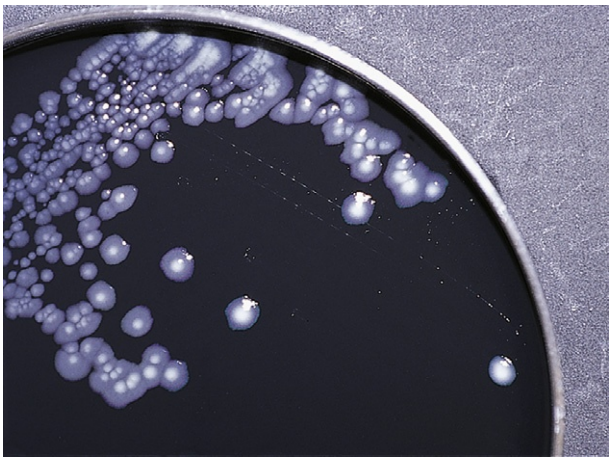


Figure 24 Growth of *Legionella pneumophila* on the enrichment medium-buffered charcoal–yeast extract (BCYE) agar, used specifically to grow this bacterial genus. Reprinted from Figure 7-2 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

Molecular Microbiology Methods

In situ *hybridization, PCR, and sequencing*

The use of molecular techniques in microbial identification has increased dramatically over the past decade and there is still a rapid rate of introduction of new and revised methods and applications. Detection of an organism's nucleic acid does not require a viable organism, which is a big advantage. Molecular methods overall tend to be very sensitive and specific. Fluorescence *in situ* hybridization (FISH) is a technique using a tagged nucleic acid probe on cultures or fixed tissue to identify the DNA of the suspected organism (Figure 34). PCR is a powerful method and is used in a number of ways. PCR uses a heat-stable DNA polymerase combined with DNA oligomers (primers) of a specific sequence designed to amplify a specific target. Multiple cycles of heating denature the DNA, followed by cooling to allow specific hybridization of primers, and then extension of synthesized product by the polymerase enzyme can result in a millionfold increase in the DNA of interest. The process can be completed in several hours or shorter with new fast instruments (Figure 35). Multiple adaptations of PCR include reverse-transcription PCR, which includes an initial step to convert RNA into DNA, followed by DNA amplification, or real-time PCR that permits quantification of the starting nucleic acid. The DNA product is measured in each cycle, often by a fluorescent DNA probe, and the amount of signal on each cycle is proportional to the starting material (Figure 36). Real-time PCR is used for cytomegalovirus, Epstein–Barr virus, BK and JC polyomaviruses, herpesviruses, and quite a few more targets. The conversion of viral testing from viral culture techniques to molecular methods had a huge impact on test turnaround time. Some viral cultures previously took 14–21 days, but results can now be obtained in a few hours. Most of virology testing has moved from viral culture, shell-vial culture,

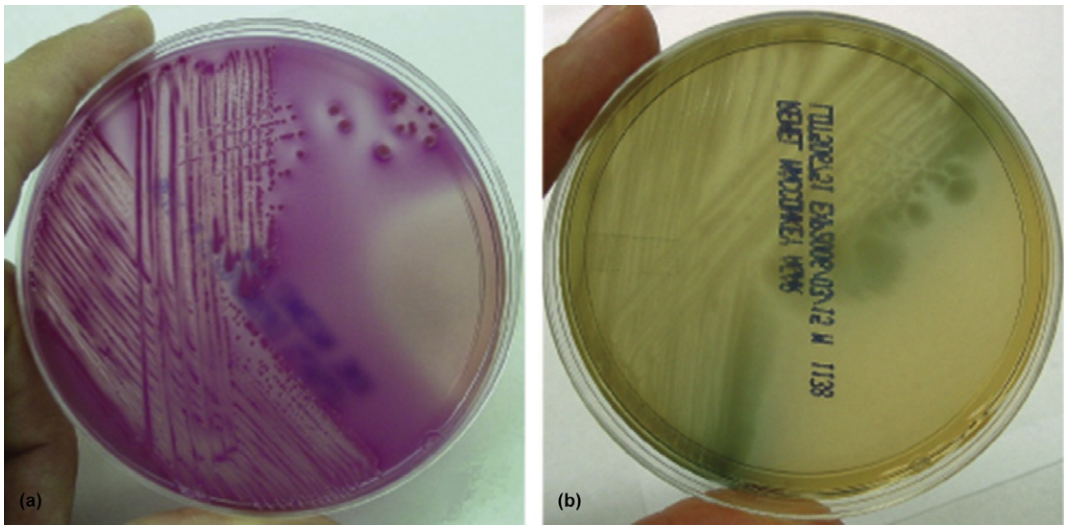


Figure 25 MacConkey agar. (a) *Escherichia coli*, a lactose fermenter, has a pink color on this differential media. (b) *Pseudomonas aeruginosa*, a non-lactose fermenter, does not create a pink color on the agar. Reprinted from Figure 5-3 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

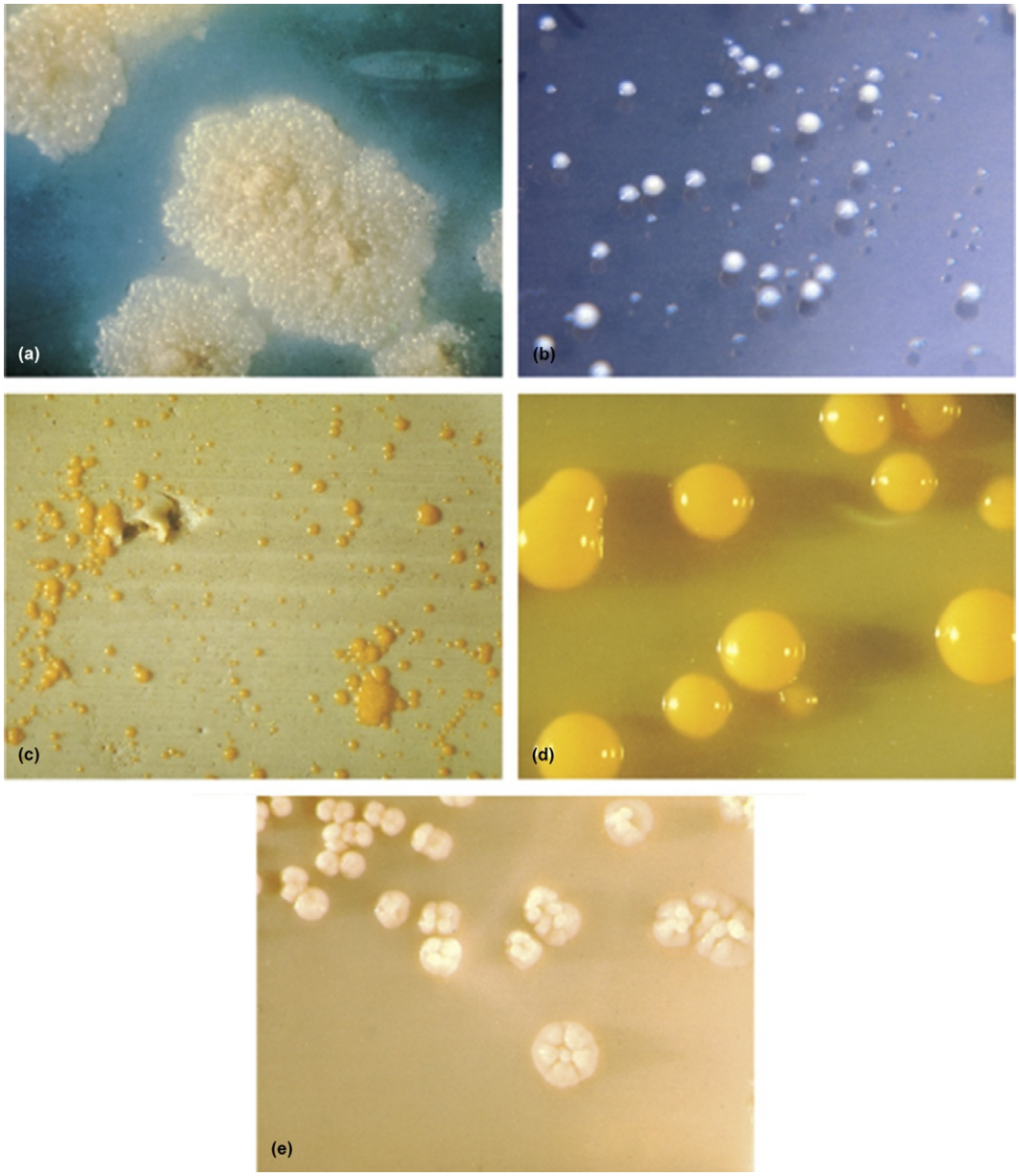


Figure 26 Typical appearance of some mycobacteria on solid agar medium. (a) *Mycobacterium tuberculosis* colonies on Lowenstein–Jensen agar after 8 weeks of incubation. (b) Different colonial morphology seen on culture of one strain of *Mycobacterium avium* complex. (c) *Mycobacterium kansasii* colonies exposed to light. (d) Scotochromogen (pigmented in both light and dark incubation) *Mycobacterium gordonae* with yellow colonies. (e) Smooth, multilobate colonies of *Mycobacterium fortuitum* on Lowenstein–Jensen medium. Reprinted from Figure 45-3 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

rapid immunochromatographic assays, and DFA assays to real-time PCR assays. Multiplex PCR includes multiple primer sets in a single reaction with a number of ingenious ways to separate and detect the signal of the specific pathogens. Assays currently may have 16 or more targets in an individual assay. Respiratory virus detection was greatly improved by multiplex methods compared to culture, DFA assays, antigen detection, and single-target PCR assays. DNA sequencing of the gene for the 16S ribosomal subunit, a component of the cellular protein synthesis machine, is used to identify bacteria that may not be identified by phenotypic methods described earlier. Sequencing ribosomal or other genes may also be used for identification of fungi, mycobacteria, and viruses.

Probes and hybrid capture assays

Nucleic acid probes have been used to identify *M. tuberculosis* and some other mycobacteria for a number of years. The probe has a chemiluminescent label and the DNA probe targets the ribosomal RNA. The resulting DNA–RNA hybrid can be distinguished from a nonhybridized probe, and a stable DNA–RNA hybrid is detected by light produced by the chemical reaction. AccuProbes (Gene-Probe) are still used, but they have

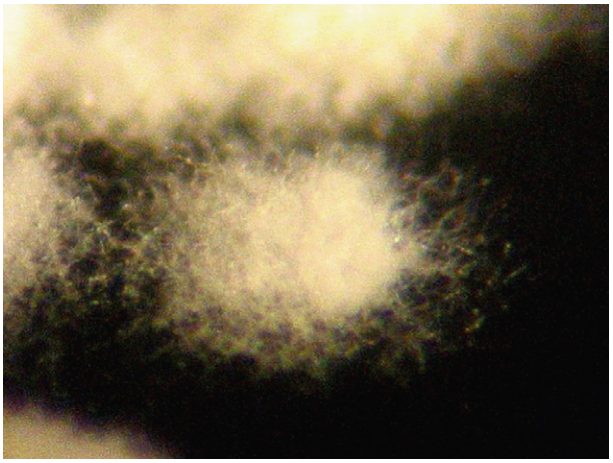


Figure 27 Aerial hyphae of *Nocardia*. Reprinted from Figure 24-4 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

been replaced by sequencing or MALDI-TOF in a number of laboratories. Hybrid capture assays are used for detection of human papillomaviruses, *Neisseria gonorrhoeae*, *Chlamydia*, and cytomegalovirus. The method involves signal amplification of RNA probes complementary to a DNA target. A monoclonal antibody conjugated to a reporter molecule may be used to detect the RNA/DNA hybrid.

Isothermal methods and branched-chain DNA assays

Additional nucleic acid amplification methods include the isothermal methods. For nucleic acid sequence-based amplification and transcription-mediated amplification, RNA is reverse-transcribed into cDNA, and RNA copies are synthesized with RNA polymerase. Loop-mediated amplification is another isothermal method that has been developed for a number of targets. Branched-chain DNA assays are an alternative to PCR. Short DNA sequences complementary to viral RNA are attached to a surface to capture the target. Next, a second cDNA is added attached to a branched chain of DNA. With subsequent cycles, the signal is amplified. This method is used for sensitive detection of HIV viral loads (Figure 37). Standard of care for diagnosis and treatment of patients with HIV infections includes the determination of actual viral load and phenotypic/genotypic determination of likely susceptibility to anti-HIV agents. Instruments for HIV testing have been approved by the Food and Drug Administration (FDA), a US federal agency responsible for protecting public health, and standardized reporting of viral titer in international units provides a comparable result for a specific specimen regardless of where in the world the specimen was tested, by incorporating a global quality control into the data analysis.

Challenges

One significant challenge with molecular methods is contamination of new reactions with amplified product from previous reactions. Assays have been developed to be 'closed' systems to avoid this problem and laboratories separate the working bench location of nucleic acid extraction of new patient specimens from the postamplification steps. Stringent controls are required to ensure ongoing quality assays.

Epidemiological Typing Methods for Outbreak Investigations

An infection control team in a hospital monitors for spread of pathogenic organisms within the hospital from one patient to another. Because hospitalized individuals are battling their underlying illnesses, they are vulnerable to severe complications from a nosocomial infection. Similarly, an outbreak of an organism in the community is tracked by public health labs to determine the source of infections and to stop an outbreak if possible. Some of the systems to investigate clonality (genetic similarity) of organisms include pulsed-field gel electrophoresis (PFGE), plasmid profiling, multilocus sequence typing (MLST), repetitive-element PCR, and genome sequencing. Restriction fragment length polymorphism (RFLP)-based methods utilize the variation in DNA fragments obtained upon a specific restriction enzyme digestion of bacterial genomic DNA. PFGE involves digestion of isolates by restriction enzymes in an agarose plug, choosing an enzyme that will result in 10–20 DNA fragments. Then, the DNA is separated by a specialized electrophoresis device that uses a pulsed electric field (Figure 38). The RFLPs are compared by analyzing multiple isolates on the same gel to determine similarity of the patterns. Plasmid profiling involves gel electrophoresis of plasmids after disruption of genomic DNA. The discriminating power of this method is variable. Repetitive-element PCR amplifies the region between interspersed repetitive DNA elements. The amplicons generated will vary between strains, thus allowing determination of strain similarity in an outbreak. MLST detects variations due to mutations or recombination by direct sequencing of fragments in housekeeping genes or another specific set of genes, such as virulence genes. The different MLST profiles of organisms under investigation are then compared. Whole-genome sequencing is now possible as an epidemiological tool. With the decrease in the cost of sequencing, entire genomes can be compared and single-nucleotide polymorphisms, deletions, insertions, or recombinations can be detected. Only a limited number of labs have access to whole-genome sequence techniques and the bioinformatics experts to analyze the data. Sequencing can also be used to compare viral strains that are implicated in an outbreak.

Serological Techniques

Serological diagnostic techniques examine the antibody response of an individual to a specific organism. A change in antibody titers to a specific microbial antigen may allow the diagnosis of an infectious disease that would otherwise be undiagnosed due to the lack of positive cultures because of our inability to culture the organism, due to prior antimicrobial therapy, or due to a lack of alternative assays that would detect the organism. Serology is useful in these cases to establish a diagnosis and to determine if the infection is acute or chronic, a primary infection or a reinfection, by analyzing for the presence of immunoglobulin (Ig) M, IgG, and the change in titer with time. Some agents for which serology is helpful include Epstein–Barr virus, hepatitis viruses, HIV, human T-cell leukemia virus, rabies virus, *Mycoplasma pneumonia*, and arboviruses. Serological monitoring is important to track successful immunization following vaccination. Serological tests for

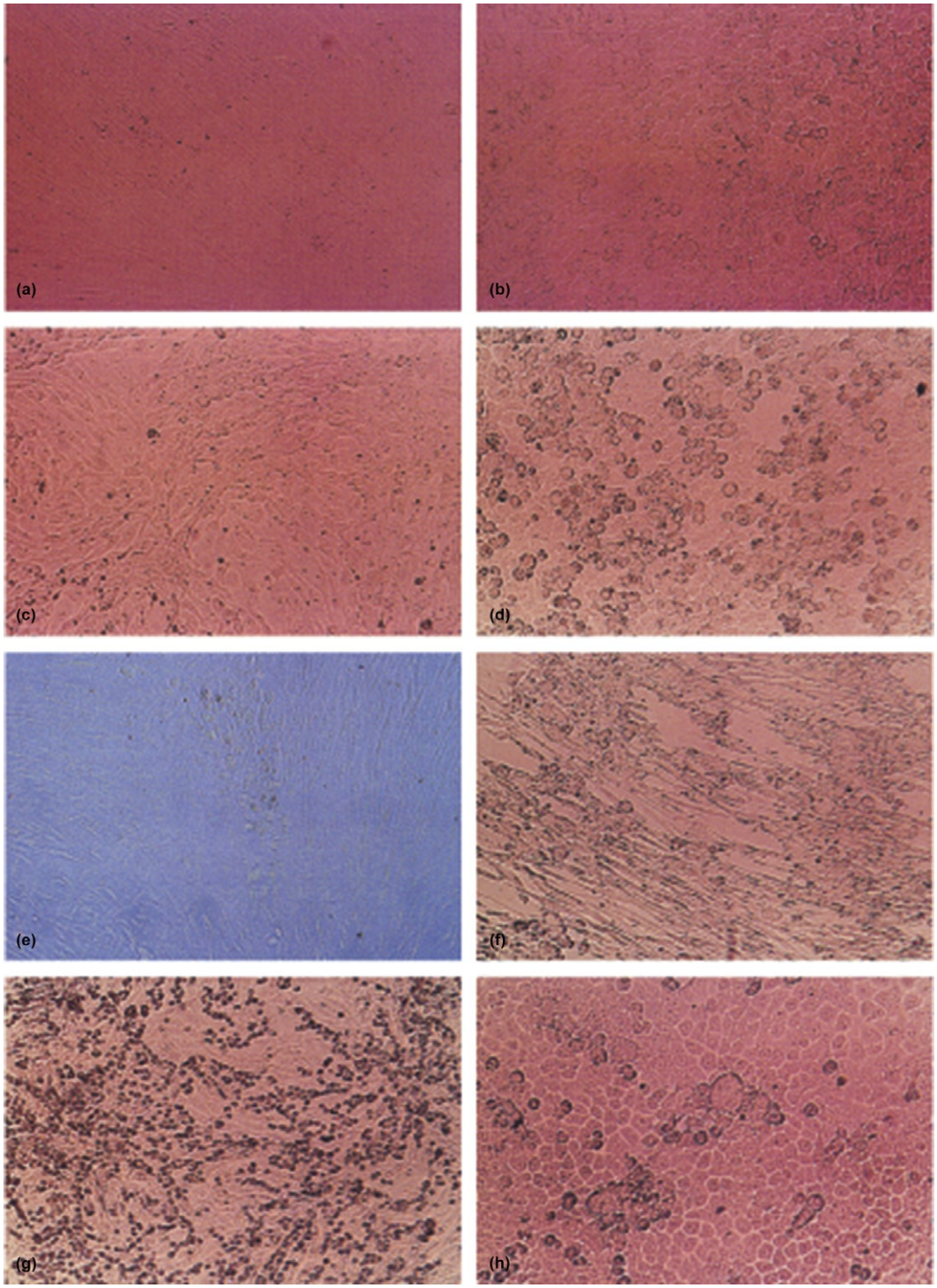


Figure 28 Cell culture morphology and viral cytopathic effect. (a) Normal human diploid lung fibroblast (HDF) cells. (b) Normal HEp-2 cells. (c) Normal primary monkey kidney (PMK) cells. (d) HEp-2 cells infected with adenovirus. (e) HDF cells infected with cytomegalovirus. (f) HDF cells infected with herpes simplex virus. (g) PMK cells infected with hemadsorbing virus, such as influenza, parainfluenza, or mumps, plus guinea pig erythrocytes. (h) HEp-2 cells infected with respiratory syncytial virus. Reprinted from Figure 51-24 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

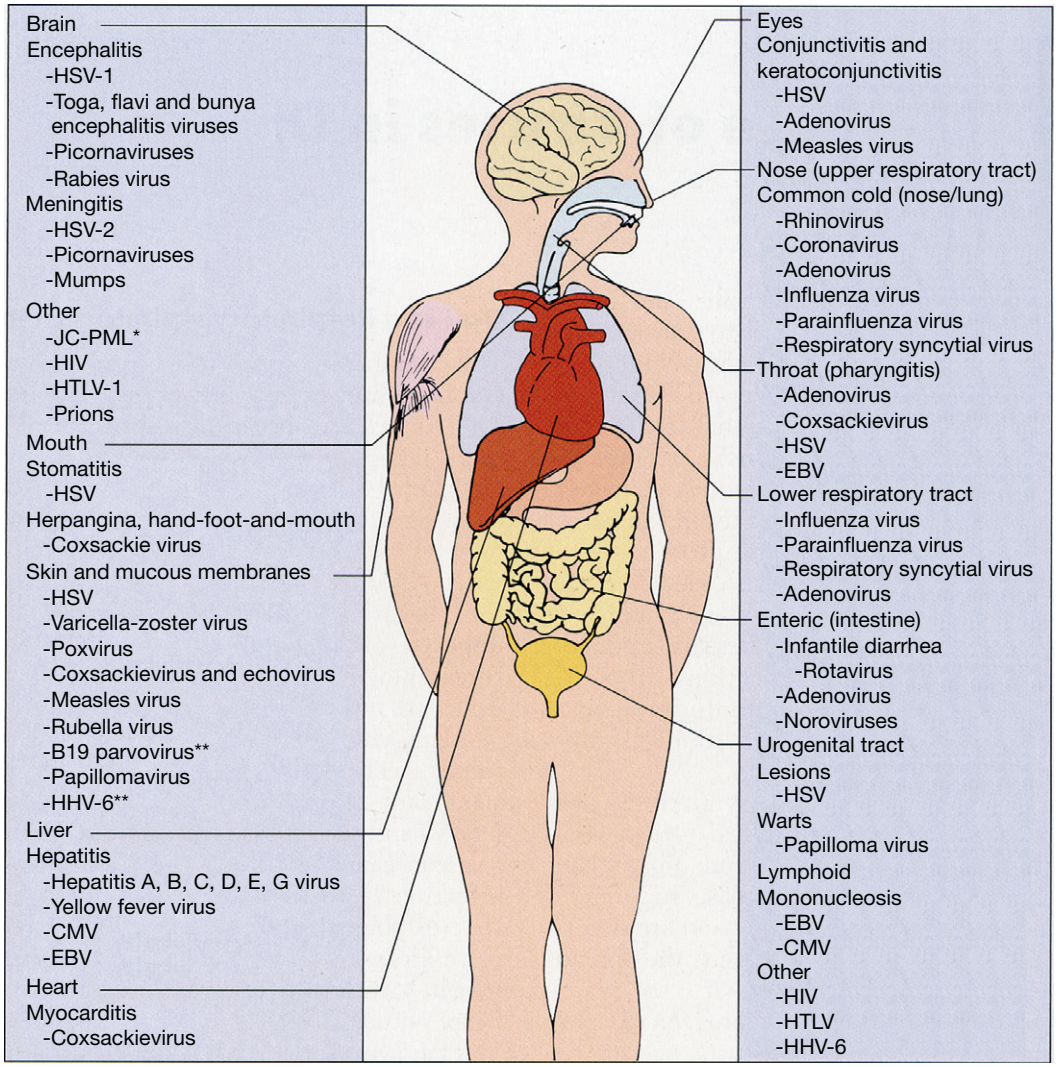


Figure 29 Major targets of viral disease. Asterisk (*) indicates progressive multifocal leukoencephalopathy. Infection by viruses indicated by double asterisks (**) results in an immune-mediated rash. CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV-6, human herpesvirus-6; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HTLV, human T-cell lymphotropic virus; JC-PML, JC papovavirus-induced progressive multifocal leukoencephalopathy. Reprinted from Figure 46-1 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

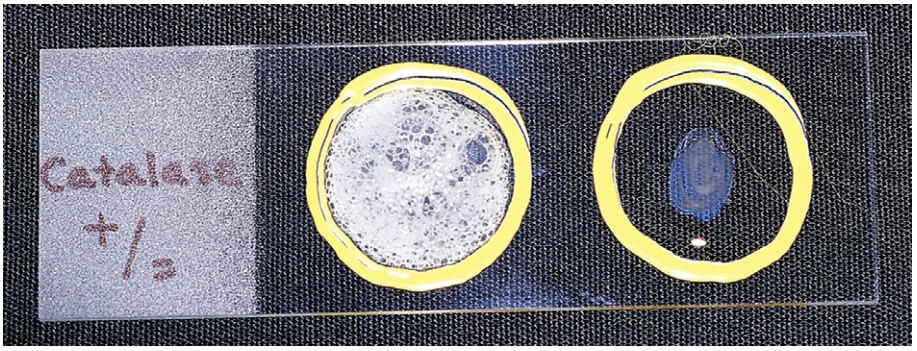


Figure 30 Rapid biochemical reaction. Catalase test. Left circle, positive. Right circle, negative. Reprinted from Figure 13-8 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

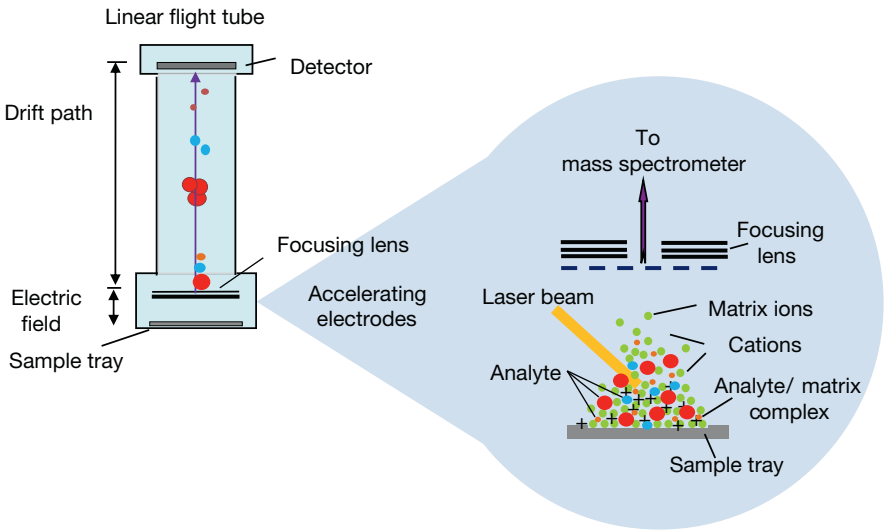


Figure 31 Principle of MALDI-TOF MS identification of bacteria and yeast in schematic diagram. Laser impact causes thermal desorption of (ribosomal) proteins of bacteria/yeast embedded in matrix material and applied to the target plate (analytes are shown in red, light blue, and orange spheres; the matrix is given as green spheres). In an electric field, ions are accelerated according to their mass and electric charge. The drift path allows further separation and leads to measurable differences in time of flight of the desorbed particles that are detected on top of the vacuum tube. From time of flight, the exact mass of the polypeptides can be calculated. Reprinted from Wieser, A., Schneider, L., Jung, J., and Schubert, S., (2012). MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond. Appl. Microbiol. and Biotechnol. 93, 965–974, with permission.

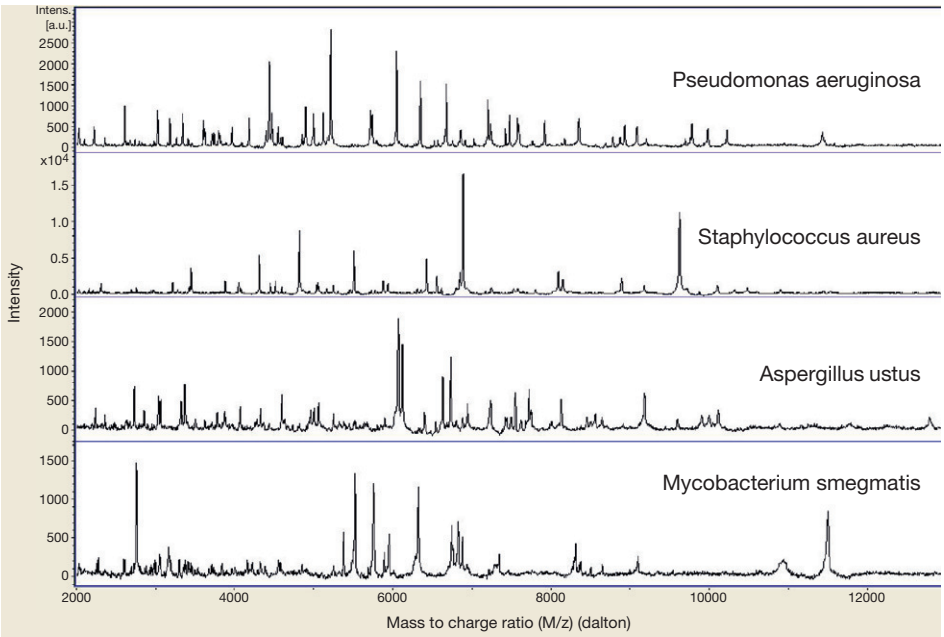


Figure 32 MALDI-TOF spectra of four organisms. The unique pattern of peaks correlates with specific species and provides a reliable identification method. Image courtesy of Anna Lau.

hepatitis B, rubella, measles, varicella zoster, and mumps viruses help document vaccine efficacy.

Susceptibility Testing

To choose the most appropriate antimicrobial agent for treatment of a patient's infection, susceptibility testing in the laboratory can be essential. Empirical therapy that is started at the time of symptoms, before a definitive diagnosis is established or before a causative pathogen is identified, is usually very broadly active, may have unwanted side effects, and may be inactive against the true infecting agent. Furthermore, widespread use of broad-spectrum antibiotics contributes to the spread of organisms that are resistant to multiple antibiotics.

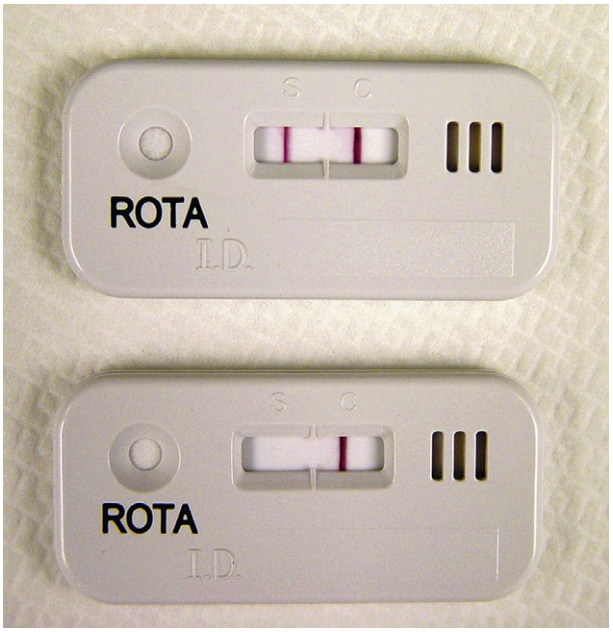


Figure 33 Positive (top) and negative membrane enzyme-linked immunosorbent assay tests for the detection of rotavirus. The red line in the reaction area on the left represents a positive test. A red line in the reaction area on the right represents an internal test control ensuring that the test has been carried out correctly. If the test control line is not present, the test is invalid and must be repeated. Reprinted from Figure 51-18 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

So for multiple reasons, it is important to identify the specific antimicrobial therapy for a specific infection. Antibacterial agents work through various mechanisms including disruption of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, inhibition of DNA/RNA synthesis, or inhibition of metabolic processes. Different organisms have developed various mechanisms of antimicrobial resistance. The susceptibility testing must be standardized for accuracy. Methods include broth dilution, agar dilution, disk diffusion, broth microdilution, E-test gradients, and genotypic methods. Various methods are illustrated in **Figures 39–43**. For each, the organism is incubated in the presence of different concentrations of the drug to determine the lowest concentration that inhibits the growth of the organism, thus determining the minimal inhibitory concentration (MIC). To determine the clinical significance of the data and to translate the MIC from the laboratory into information that the physician can use to determine if the drug will likely be effective to treat a specific patient, guidelines have been established by the Clinical and Laboratory Standards Institute. Some susceptibility testing methods are manual and labor-intensive. However, multiple automated instruments have been developed to test antimicrobial susceptibility in broth culture, detect the MIC, and interpret the MIC. Expert software has been written to check for unusual patterns that may indicate an error in organism identification or resistance patterns that signal a multidrug-resistant organism. A separate approach to susceptibility testing is the detection of a resistance gene with molecular methods. These methods can be much more rapid than phenotypic testing. Examples of common genotypic testing include the mecA gene used to detect MRSA and the vanA gene used to detect vancomycin-resistant *Enterococci*. Sophisticated phenotypic and genotypic testing of HIV is performed routinely by specialized laboratories for optimal choice of highly active antiretroviral therapy. Because host factors and the organ infected can influence the effectiveness of the chosen therapy, patient symptoms must be monitored closely and therapy adjusted if the patient is not improving on the drug to which the organism was reported to be sensitive.

Test Interpretation

Interpretation of Microbial Identification

All microbiological test results must be interpreted in the context of the patient. Each organism identified in a specimen may be a pathogen, a component of commensal flora, or an environmental contaminant. The underlying condition of the patient will strongly influence the interpretation of the laboratory data. Patients who are immunocompromised due to autoimmune diseases, cancer chemotherapy, HIV infection, immunomodulating therapy, or genetic disorders that result in immunocompromise are more likely have true infections from an opportunistic pathogen than an otherwise healthy, immunocompetent host. Molecular methods may detect DNA of a nonviable organism or detect a very low burden of organism that is not responsible for the symptoms of the patient. Interpretation will be affected by the age of the patient. Newborns, children, and adults have different pretest probabilities of some specific causes of infection. For example, meningitis in a newborn is more likely to be due to *Escherichia coli* or group B *Streptococcus*, whereas meningitis in an older patient may be due to *Neisseria meningitidis* or enterovirus. Contamination of cultures with organisms that are potential pathogens can complicate interpretation of results. If an environmental organism that has never been reported to cause human disease is isolated, it is almost certainly a contaminant. One would need to do an extensive investigation to confirm that a new organism was responsible for disease. In some cases, based on the colony location on a nutrient agar plate, it is easy to determine if an isolate is a laboratory contaminant. In other cases, it is much more difficult, so contamination must be kept to an absolute minimum with strict sterile technique when processing and working up cultures. Another important feature of interpretation may be the quantity of organism in the specimen. A small amount of a potential pathogen in a culture that is mixed with multiple other bacteria may suggest that the organism is a colonizer, whereas a heavy growth of a single organism type is more likely a pathogen. A urine culture is an example of a routine quantitative culture that is interpreted based on the mixture and quantity of organisms present. In some cases, lower respiratory tract or wound cultures may be quantitative. It is essential to know the list of normal flora for each specimen source when interpreting results. Identification of an organism from a sterile site is almost always significant, with the exception of contaminated specimens. For phenotypic methods, it is not always possible to identify an organism with absolute certainty. A lab may report a result as 'most consistent with organism X.' This occurs partly

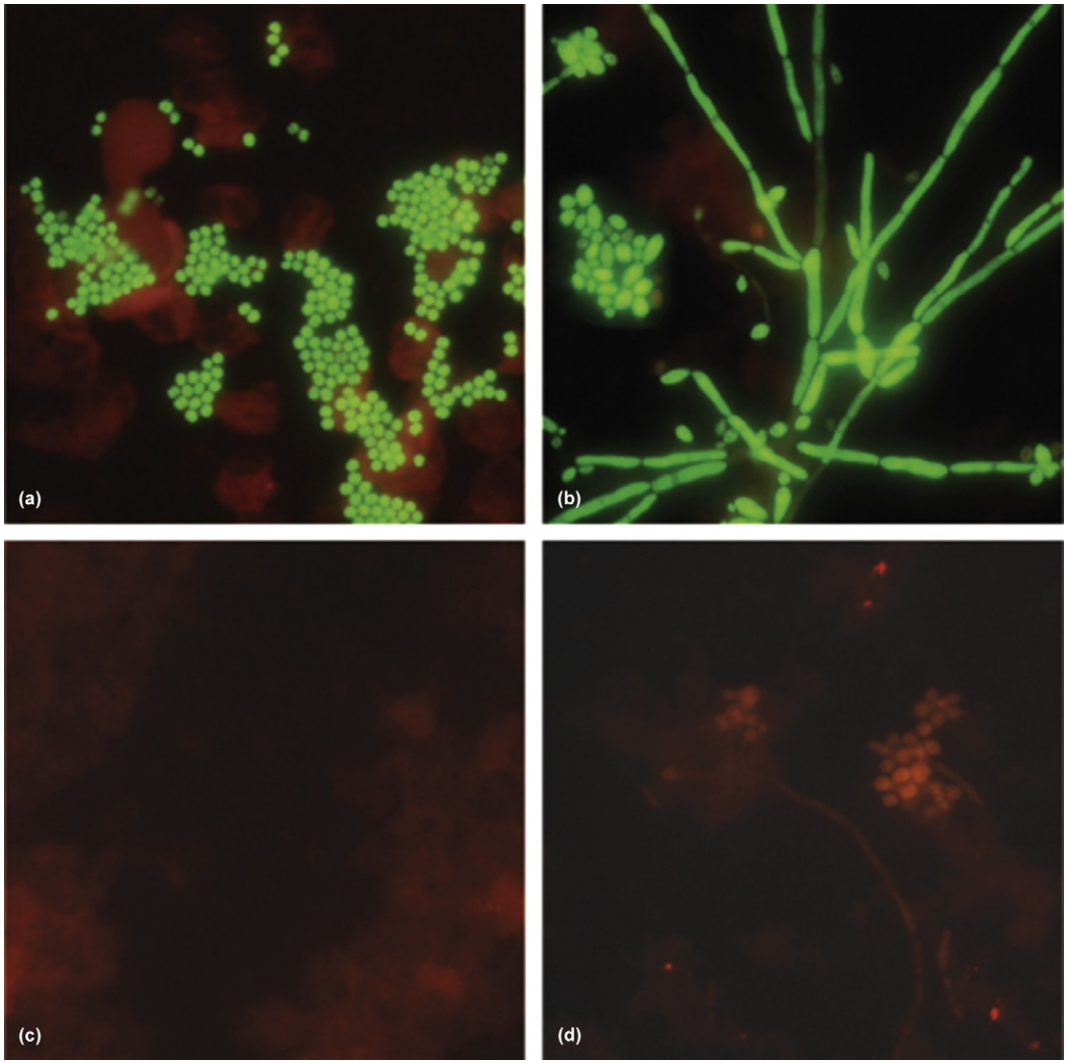


Figure 34 Using a fluorescent-tagged peptide nucleic acid (PNA) probe in conjugation with fluorescent *in situ* hydridization (FISH), *Staphylococcus aureus* (a) or *Candida albicans* (b) was directly identified in blood cultures within 2.5 h. A drop from the positive blood culture bottle is added to a slide containing a drop of fixative solution, which keeps the cells intact. After fixation, the appropriate fluorescent-labeled PNA probe is added. The PNA probe penetrates the microbial cell wall and hybridizes to the rRNA. Slides are examined under a fluorescent microscope. If the specific target is present, bright green, fluorescent-staining organisms will be present. Blood cultures negative for either *S. aureus* (c) or *C. albicans* (d) by PNA FISH technology are shown. Courtesy AdvanDx, Woburn, MA. Reprinted from Figure 8-6 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

because not every organism of a specific species may give the same enzymatic result for one test. Likewise, for sequencing and MALDI-TOF results, the data may not be adequate to distinguish between several species, and several possible organisms may be reported. When a specimen is cultured in the clinical lab, results are often available in a series of steps. The direct Gram stain is known immediately, and then isolates grown from the culture may be evident in 12, 24, 48, 72 h, or longer. A culture might have several microorganisms evident on different days. The treating physician receives preliminary reports, followed by updates as they become available. The clinician, with the aid of the clinical microbiologist, must create a differential diagnosis including the list of organisms that fit the data to date and then treat the patient with antimicrobials to cover possible organisms. They must weigh the risks and benefits of narrowing therapy at any given time, balancing the negative aspect of toxicity of several broad-spectrum agents against the benefit of covering all possible likely pathogens until the culture results are finalized. The severity of illness of the patient will greatly impact these decisions.

Interpretation of Positive Blood Cultures

Typically, about 3–7% of positive blood cultures are due to contamination during the collection process. Several factors can be used to assess the clinical significance of the positive blood culture: (1) organism identification, because skin flora are frequent contaminants whereas other organisms are rarely contaminants; (2) more than one positive blood culture with the same organism increases the likelihood of a true positive finding of bacteremia, bacteria in the blood that may be causing disease as opposed to a false-positive result; and (3) growth

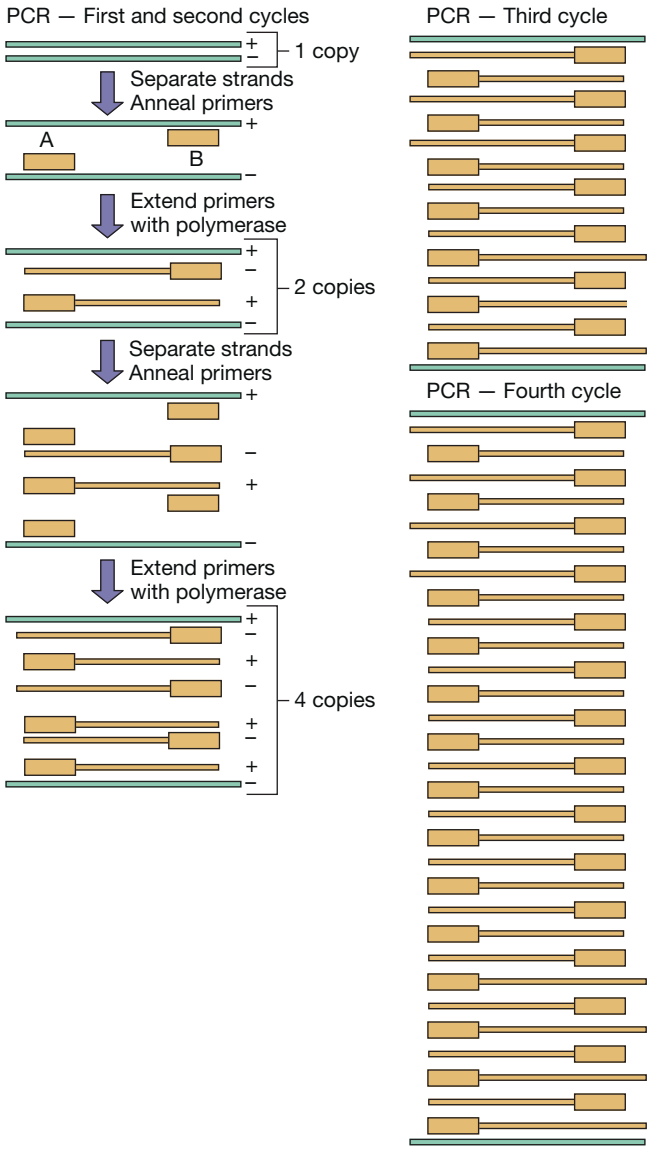


Figure 35 Polymerase chain reaction (PCR). This technique is a rapid means of amplifying a known sequence of DNA. A sample is mixed with a heat-stable DNA polymerase, excess deoxyribonucleotide triphosphates, and two DNA oligomers (primers), which complement the ends of the DNA, and then cooled to allow binding of the primers to the target DNA and extension of the primers by the polymerase. The cycle is repeated 20–40 times. After the first cycle, only the sequence bracketed by the primers is amplified. In the reverse transcriptase PCR technique, RNA can also be amplified after its conversion to DNA by reverse transcriptase. Labels A and B, DNA oligomers used as primers; + and −, DNA strands. Modified from Blair, G.E., Blair Zajdel, M.E., 1992. Biochem. Educ. 20, 87–90. Reprinted from Figure 5-4 of Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA, with permission from Elsevier.

of the organism in both the blood and another sterile or potentially infected site in the same patient indicates likely bacteremia and disease. *Corynebacterium* species, *Bacillus* species, and *Propionibacterium* species are most often contaminants. The isolation of *S. aureus*, *E. coli*, Enterobacteriaceae, *P. aeruginosa*, *S. pneumoniae*, or *Candida albicans* from blood cultures is almost always clinically significant.

Interpretation of a negative microbiological result

A negative result from a microbiological culture, PCR assay, fluorescent antibody assay, or other detection method could mean that the patient does not have an infection with that organism, or that the specimen was inadequate, or that the assay is not sensitive enough. Some organisms are much harder to culture than others, or to detect by alternative methods such as antigen detection, special stains, or serological testing. In some situations, multiple assays will be used simultaneously to rule out an infection, such as cultures from multiple sites, combined with a molecular assay, combined with serological testing. Cost considerations would favor limiting testing to the most appropriate number of tests needed for a given patient. Likewise, any assay has some number of false-positive results, so all results must be considered in a clinical context.

Interpretation of Quantitative Molecular Results

Caution must be used when interpreting quantitative molecular results, such as viral loads. Many of the tests used in clinical laboratories are not available as FDA-approved assays and the results are not standardized, although all laboratories are required to perform extensive validation of their assays. While HIV viral load testing is standardized, many viral assays are developed in-house by specific laboratories and are not FDA-approved. The viral loads for cytomegalovirus, Epstein–Barr virus, and adenovirus are often followed over time in transplant patients. The comparison of viral loads over time in a single patient should be done with data from a single accredited laboratory. However, a result from one laboratory should not be compared to a result from a second laboratory to determine if the viral load has increased or decreased, unless the results are standardized between laboratories. Otherwise, the physician may mistakenly determine that the viral load has changed for better or worse when it has not.

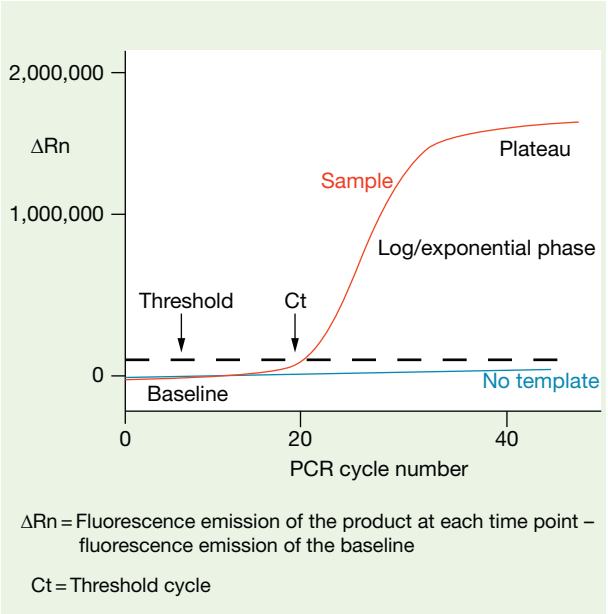


Figure 36 Model of a single amplification plot illustrating the nomenclature commonly used in real-time quantitative PCR. Reprinted from Arya, M., Shergill, I.S., Williamson, M., Gommersall, L., Arya, N., and Patel, H.R.H., 2005. Basic principles of real-time quantitative PCR. Expert. Rev. Mol. Diagn. 5, 209–219, with permission.

Interpretation of Gram Stains

Although Gram stains are used daily in the laboratory and provide information rapidly, judgment is required when reporting the result. There are some limitations to the assay due to variations in organism staining characteristics. For example, *Acinetobacter* is a Gram-negative coccobacillus that can be mistaken for Gram-negative coccus, such as *Neisseria*, or Gram-positive coccus, such as *Streptococcus*. Anaerobic Gram-positive organisms can frequently stain as Gram-negative organisms. A single organism type may have variations in shape, appearing as a diplococcus and also as an elongated coccus that resembles a short bacillus in the same slide, leading a microbiologist to mistakenly interpret the slide as having a mixture of two organisms. The common pitfalls are known and should be communicated from the laboratory to the treating clinician when appropriate.

Limitations

Limitations in Sensitivity of Detection

Culture was traditionally the gold standard for detection of the microbes causing infectious diseases; however, a number

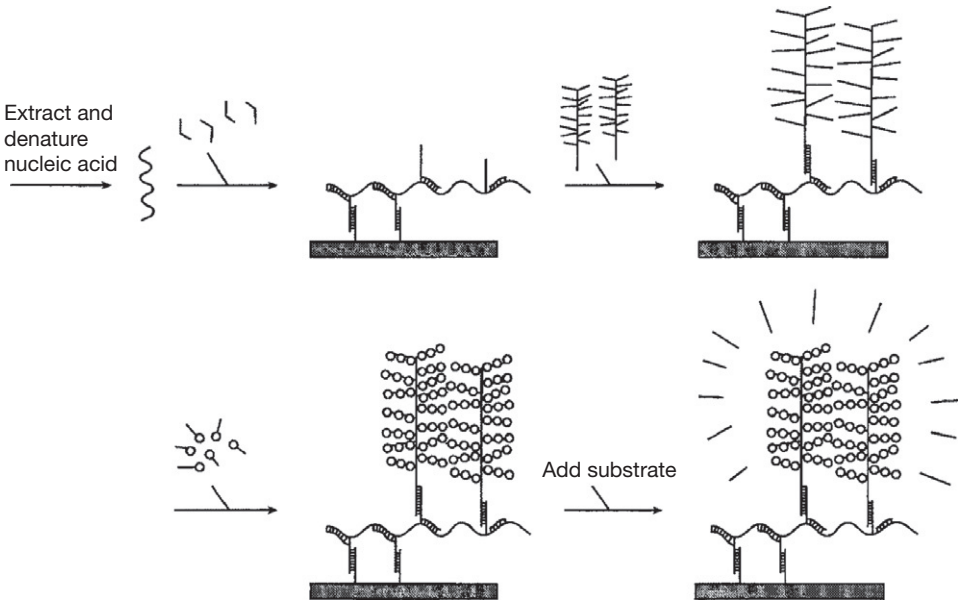


Figure 37 The principle of the branched DNA signal amplification assay. Note the signal and not the target amplification, making accurate quantitation possible. Reprinted from Lisby, G., 1999. Application of nucleic acid amplification in clinical microbiology. Mol. Biotechnol. 12, 75–99, with permission.

of organisms are either fastidious and thus difficult to culture or uncultivatable in routine laboratories. Examples include Epstein–Barr virus, *Treponema pallidum*, *Rickettsia*, and human metapneumovirus. Advances in molecular technology have been of great value in this area, expanding the number of bacteria and viruses that we can detect. However, molecular testing does not always distinguish colonization from disease-associated infections, and we can design primers and probes for molecular assays only after we have sufficient unique sequence data. Serology, as discussed, also assists in the diagnosis of organisms that we cannot grow. Testing in the clinical lab is optimized for human pathogens. Environmental organisms may not grow. Bacteria, viruses, or fungi that are not known to infect humans are not included in the assay design. Emerging infectious agents may not be detected quickly for this reason.

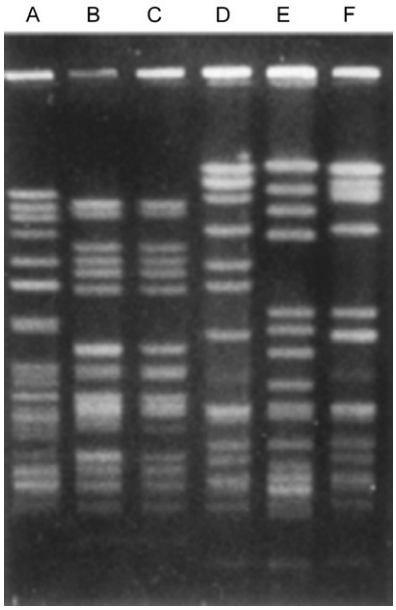


Figure 38 Although antimicrobial susceptibility profiles indicated that several methicillin-resistant *S. aureus* isolates were the same strain, restriction fragment length polymorphism analysis using pulsed-field gel electrophoresis (lanes A through F) demonstrates that only isolates B and C were the same. Reprinted from Figure 8-17 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

Limitations of Turnaround Time for Results

Unlike many chemistry tests that can be run immediately, directly from a blood specimen within minutes, microbiology results are often delayed due to the time required to grow an organism, isolate it from the other organisms in the culture, and determine susceptibility results. One approach to reduce turnaround time for blood cultures is the use of FISH, which uses peptide nucleic acid probes targeting specific microbe 16S rRNA after a blood culture has flagged positive. A limitation is that the FISH probes will detect only organisms that are targeted, missing organisms not targeted with the selected set of probes. Although faster than traditional bacterial identification systems that require incubation time for enzymatic or carbohydrate utilization assays to occur, there is extra work to run the FISH and then to perform further testing when required. Workflow and cost considerations prevent some labs from adopting this approach. Another rapid approach is to perform MALDI-TOF analysis directly from extracted material from a positive blood culture, without waiting for isolation of specific organisms. Of significant note, organisms will often be missed in mixed infections for a number of these rapid approaches, so a complete workup of all organisms present in a blood culture is still required. The positive outcome of these rapid approaches is that preliminary data are provided to clinicians for rapid refinement of the differential diagnosis and possible early treatment changes. Scientists continue to work on the 'Holy Grail' of complete microorganism identification directly from patient specimens without the need for culture. Currently, to detect the maximum number of organisms, a routine

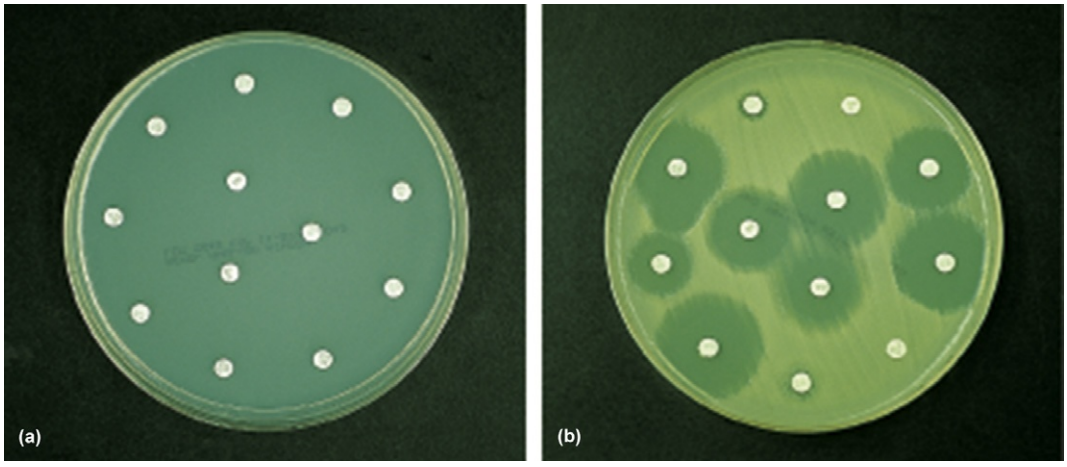


Figure 39 (a) By the disk diffusion method, antibiotic disks are placed on the surface just after the agar surface was inoculated with the test organism. (b) Zones of growth inhibition around various disks are apparent following 16–18 h of incubation. Reprinted from Figure 12-5 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

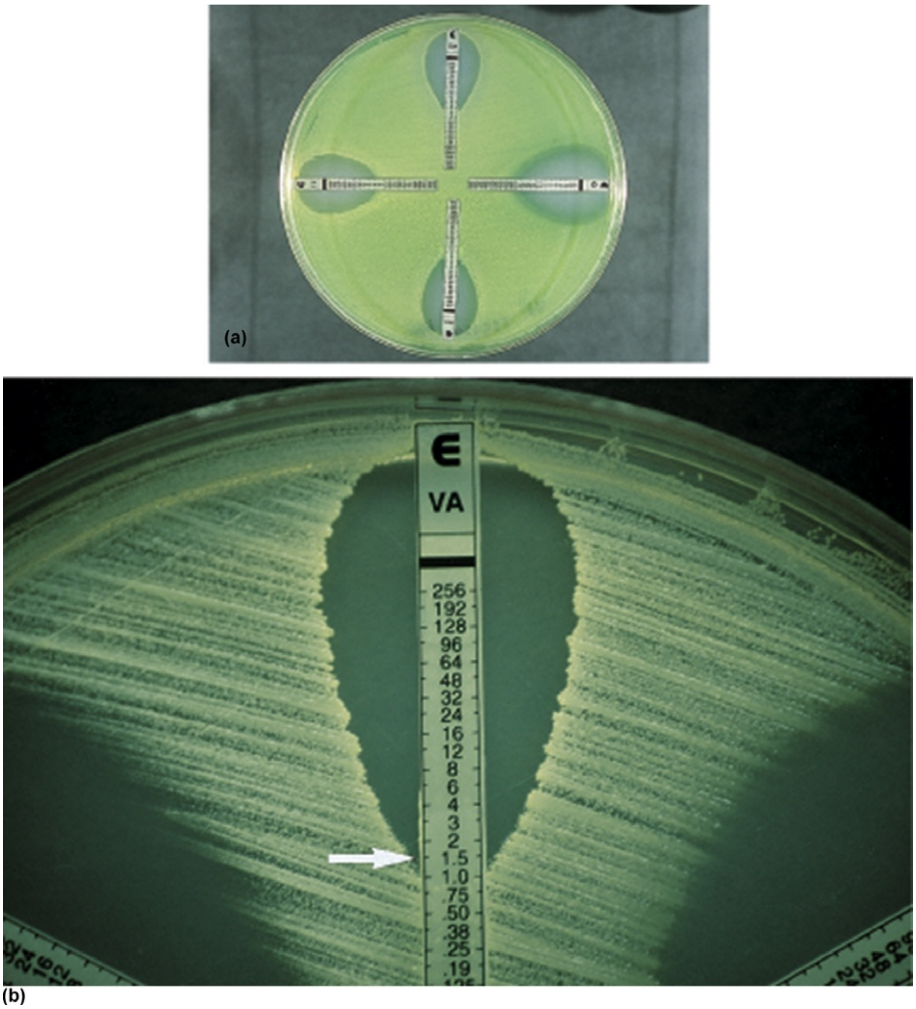


Figure 40 E-test uses the principle of a predefined antibiotic gradient on a plastic strip to generate an MIC value. It is processed like the disk diffusion. (a) Individual antibiotic strips are placed on an inoculated agar surface. (b) After incubation, the MIC is read where the growth/inhibition edge intersects the strip graduated with the MIC scale across 15 dilutions (arrow). Several antibiotic strips can be tested on a plate. Courtesy AB BIODISK, Solna, Sweden. Reprinted from Figure 12-11 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

clinical microbiology laboratory uses a combined approach of culture, serology, antigen detection, biomarker detection, molecular methods, microscopy, and stains.

Limitations of Susceptibility Testing

The thresholds defined for the interpretation of a specific bacterial isolate as sensitive or resistant to a specific drug, such as the susceptibility of *Klebsiella* to ceftazidime, are based on guidelines from the Clinical and Laboratory Standards Institute (CLSI). The CLSI is a consensus body created to make decisions on best available data, including pharmacokinetic/pharmacodynamic data, animal models, *in vitro* testing, computer simulations, and limited patient outcome data. When there are multiple options for therapy because an organism tests sensitive to multiple drugs, the method generally works well. But when an organism is resistant to many drugs, then the best option clinically is not always clear. Within the past several years, the guidelines provided by CLSI have changed the cutoff values for the MIC value that should be translated into sensitive, intermediate, or resistant for a number of organism/drug combinations. Because the FDA approval of microbiology equipment is independent of the CLSI process, this resulted in different labs using different cutoffs based on which FDA-approved instrument or method they used. This issue strongly highlights the fact that medicine is still both an art and a science, as a physician must incorporate all signs, symptoms, and test results to make the best therapeutic plan.

Database Limitations

Databases to interpret biochemical reaction profiles of bacteria, DNA sequence databases, or mass spectrometry databases to interpret proteomic profiles from MALDI-TOF methods must be updated routinely, and laboratory staff must be aware of which organisms are included in the database.

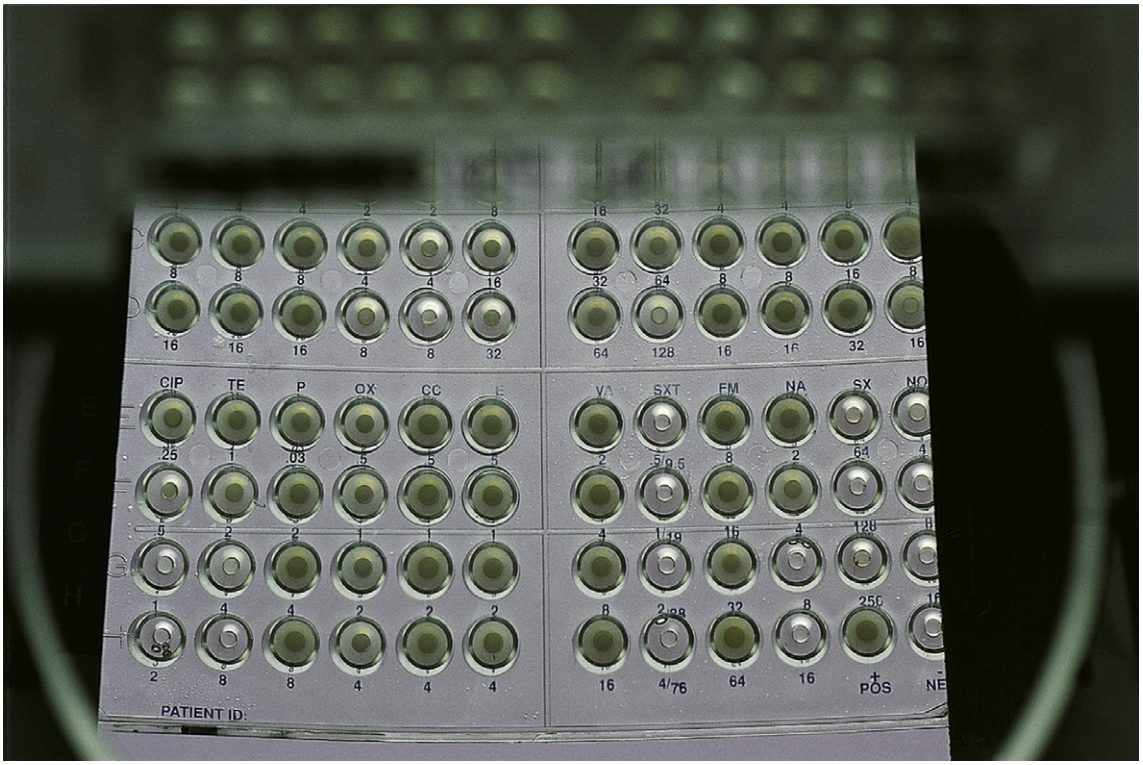


Figure 41 Bacterial growth profiles in a broth microdilution tray. The wells containing the lowest concentration of an antibiotic that completely inhibits visible growth (arrow) are recorded, in μg ml^{-1}, as the minimal inhibitory concentration (MIC). Reprinted from Figure 12-3 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

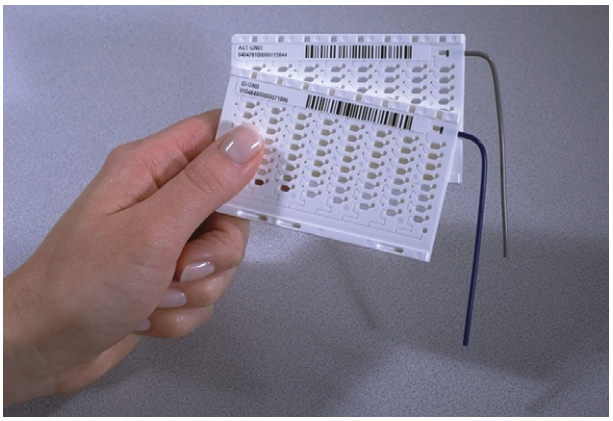


Figure 42 The VITEK 2 antimicrobial susceptibility test card contains 64 wells with multiple concentrations of up to 22 antibiotics. The antibiotic is rehydrated when the organism suspension is introduced into the card during the automated filling process. Courtesy bioMérieux, Inc. Hazelwood, MO. Reprinted from Figure 12-12 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

When a specific species is not included in the database, it cannot be selected; therefore, misidentification has resulted from this limitation in the database. Nomenclature also changes, so databases and reports must be updated accordingly.

Contamination of Molecular Assays

A limitation of many molecular assays involving an amplification step is the possible contamination of the work area and patient samples with amplified product. There have been many improvements to address this concern, such as closed tubes and more automated instruments that reduce the likelihood of contamination. Also, addition of deoxyuridine triphosphate (dUTP) instead of thymidine triphosphtate (dTTP) to the reaction mix, combined with the use of uracil DNA glycosylase prior to each amplification step, has been beneficial in eliminating contaminating amplicons from previous amplifications. Finally, awareness of the issue and inclusion of negative controls is essential to keep this problem to a minimum.

Cost

A significant concern in most hospitals is the cost of patient care, a component of which is the cost of laboratory testing. As our technology advances in the microbiology laboratory and we are able to test for more microbes, the cost is often much higher than traditional method costs. It is now more common to create a panel of pathogens that are tested by a molecular method at one time instead of culture and assays that focus on each potential pathogen individually, based on likelihood of the presence of that pathogen. The panels may include common pathogens along with less common pathogens and

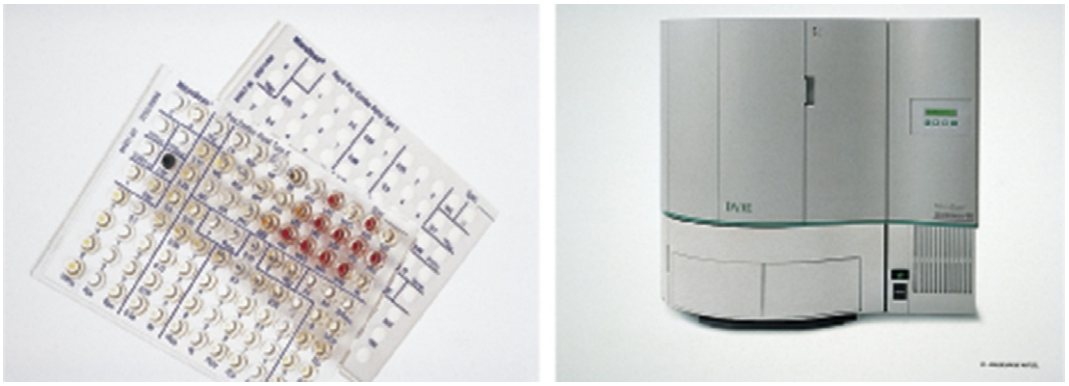


Figure 43 Microbroth tray format (a) used with the MicroScan WalkAway instrument (b) for automated incubation, reading, and interpretation of antimicrobial susceptibility tests. Courtesy Dade International, Sacramento, CA. Reprinted from Figure 12-14 of Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis, with permission from Elsevier.

the test cannot be adapted to test only a subset of targets. This creates a challenge for the ordering clinician and the microbiology director to provide the best care for each individual patient without losing sight of the budget limitations for the facility overall. It is hoped that continued research and advances in technology will result in new ways to reduce the cost of these new comprehensive assays without compromising quality and spectrum of targets.

Future

Technology Development

Nucleic acid testing will continue to become even more extensively utilized. Academic, reference, and research laboratories continually develop assays for the detection of microbes not currently available in commercially marketed products. There will likely be an increase in availability and use of international standards so that results can be reported in comparable international units, as implementation of such units for a number of viral assays has lagged behind their use in other areas, such as for coumadin levels and HIV viral loads.

Whole-genome sequence analysis has now been used during investigations of several outbreaks to rapidly and precisely define the chain of transmission of an outbreak organism. Furthermore, the lineage of the organism can be identified, and the mechanism of increased virulence compared to similar nonoutbreak strains can be determined. The power of this technique is identifying the entire nucleotide sequence of the organism, which includes orders of magnitude more data than was provided by older methods of epidemiological analysis such as PFGE. For organisms that present very different patterns of bands from a PFGE analysis, the genome analysis is not necessary; however, when a single largely clonal strain is present nationally or globally, the discriminatory power of detecting subtle changes by genome analysis may be desired to detect the spread of a hospital strain within a specific setting from patient to patient. Most routine labs do not yet have the equipment or bioinformatics expertise for this approach and usually do not need this approach for routine work outside of outbreak investigations. However, in the not-too-distant future, many hospital infection control practitioners may find this approach feasible and useful, perhaps in conjunction with online bioinformatics expertise.

An example of the power of current technologies is illustrated by the 2011 European outbreak of *E. coli* in sprouts. The infections resulted in a high percentage of patients developing hemolytic uremic syndrome, which can result in kidney failure and death. Multiple research teams sequenced the genome of the new strain very rapidly. This allowed tracking of the outbreak and determination of the changes in the genetic components of this organism that were responsible for the increased virulence.

Development of methods for detection of microorganisms directly in patient specimens without the need for culture is one goal for improving rapid diagnostics. A diagnostic instrument, T2MR (T2 Biosystems) currently under evaluation, utilizes a multistep approach of PCR for specific fungi, followed by hybridization of the amplified DNA to nanoparticles attached to capture probes. Hybridization yields microclusters detectable by T2 magnetic resonance. Initial reports indicated the method can detect yeast bloodstream infections with only one colony-forming unit per milliliter with a time of 3 h from collection to detection. The technology has the potential to greatly improve patient care through more rapid diagnosis of yeast bloodstream infections and possibly other infections, as the technology is adapted.

The introduction of mass spectrometry into the clinical microbiology laboratory is a revolutionary change in the way diagnostic microbiology is practiced. For many years, the phenotypic appearance of the organism on a selective or differential agar plate, along with biochemical assays to detect carbohydrate utilization and enzymes within the organism defined the methods of identification. As more laboratories adopt the newer methods, the phenotypic and biochemical data become much less important. Mass spectrometry methods are being utilized in more routine clinical laboratories and can now be used for bacteria, mycobacteria, and fungi. Investigators are currently exploring methods to use mass spectrometry for strain typing and susceptibility testing, but there is more work to be done in this area, which has not yet been shown to have enough discriminatory power for use in patient care.

PCR-electrospray ionization mass spectrometry is another technology that has large potential for the future of microbiological diagnostics. This is a method capable of identifying nearly all human pathogens of different types without requiring the establishment of a predesigned panel of candidate microbes for testing. This is a unique feature because most assays test for the presence of specific organisms. The method combines a PCR step with a mass spectrometry time-of-flight analyzer to identify the nucleic acid base composition of the PCR products produced in a multiplex PCR reaction. Resulting nucleotide sequence must be analyzed against a reference database of genomic sequences to identify the organisms present. This technology is primarily used for research, food testing, public health surveillance, or biopharmaceutical testing at this time and has not yet been adopted into the routine clinical microbiology laboratory. There is powerful potential in this technology, but it is not yet clear if the practical issues of cost, workflow, and technical expertise of the routine laboratory will inhibit the adoption of this methodology in many laboratories.

Public Databases

A group of scientists with expertise in genomic analysis of microorganisms has created a collaborative effort to create a Global Microbial Identifier, described on their website: http://www.globalmicrobialidentifier.org. The objective of this collaboration is to create a global system to aggregate, share, mine, and translate genomic data for microorganisms in real time, enabling a direct link from end users in academia, industry, and government (e.g., clinicians, veterinarians, epidemiologist, and microbiologists) to main databases through user-friendly platforms. This system may include a reference database that could be accessed both for single clinical tasks (simple microbiological identification) and for national and international public health surveillance and outbreak investigation and response. If the vision of this group is fulfilled, the speed and simplicity of analyzing genomic data and of putting new findings into the appropriate global context will improve dramatically.

Emerging Infectious Diseases

Given the dynamic and rapidly evolving nature of medical microbiology, there is no doubt that new pathogens will be identified over the next 5 years, and our understanding of currently known microbes, along with their associated diseases and therapies, will also change dramatically within that time frame. It is an exciting field and one that requires constant vigilance. Emerging infectious diseases assure us that there will be an ongoing need for professionals who can develop new diagnostic tests for newly identified pathogens and implement routine testing of patients. The ease of international travel by huge numbers of people easily converts what might have been a local or regional infectious disease outbreak to a global one. The CDC list about 50 emerging infectious diseases is monitored and studied in order to understand their pathogenesis, detect them as they emerge in new locations, offer guidance to practitioners who recognize the symptoms, and contain them when possible. A few examples of diseases that have emerged in recent times are included here to illustrate the importance of following these fascinating human diseases.

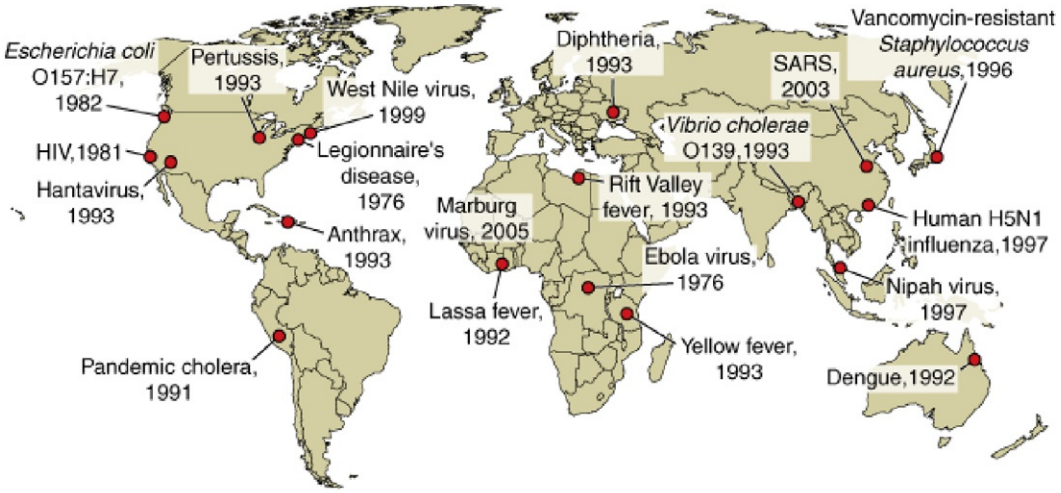
Variant Creutzfeldt–Jakob disease is a transmissible spongiform encephalopathy caused by a transmissible proteinaceous particle called a prion, first recognized in the 1990s. The disease in humans is believed to be due to exposure to bovine spongiform encephalopathy transmitted to humans from cattle. The disease is quite rare, but frightening, as there is no current cure available and the idea of a pathogenic transmissible protein that is not viral or bacterial or similar to any previously known microbes has led to much controversy over many years and much excellent research into disease mechanisms.

Extensively drug-resistant tuberculosis is a form of tuberculosis caused by mycobacteria that are resistant to some of the most effective antituberculosis drugs. Treatment is quite prolonged and can result in considerable side effects from the second- and third-line drugs used to treat the disease. This disease is associated with a much higher mortality rate than other forms of tuberculosis.

Advanced immunodeficiency syndrome due to HIV was first clinically observed in 1981 in the United States. Scientific study has now shown that HIV entered the human population at a much earlier date and is thought to have jumped the species barrier on several independent occasions, giving rise to three groups of the virus. Over the past several decades, many millions of people have died in the global epidemic of HIV; however, amazing advances in directed antiviral drugs and in diagnostic platforms have permitted many millions to live for years with the disease.

SARS, caused by a coronavirus, spreads to more than two dozen countries, infecting about 8000 people, before the global outbreak of 2003 was contained. Mortality from the disease was ~10%. Middle East respiratory syndrome is a more recent viral respiratory illness due to a related coronavirus, with initial mortality above 50%. Investigators are working to better understand the risks of this virus, including the source, how it spreads, and how infections might be prevented.

Influenza viruses adapt rapidly, and every year, scientists design a new influenza vaccine to combat the next wave of viruses. Avian influenza viruses have evolved the ability to infect humans on a number of occasions. Avian influenza A H5N1 virus has been detected for a number of years, and H7N9 virus is one of the most recently reported. Exposure to infected poultry is associated with spread to human populations. Again, avian viruses tend to cause severe disease and high mortality. Vigilant monitoring to detect adaption of one of these viruses to a strain that can be transmitted easily between humans, increasing the likelihood of a pandemic, is a high priority for public health agencies. *K. pneumoniae* that produce an enzyme known as a carbapenemase are responsible for the spread of drug-resistant Gram-negative bacterial infections to many countries in the world. In some cases, the organisms are resistant to all available drugs for Gram-negative organisms, and again, high mortality is seen in infected patients with comorbidities. Strong infection control measures are essential because the organism can be spread by contact with the environment on incompletely washed hands of healthcare workers (**Figure 44**).



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

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Figure 44 Map of the world showing examples of geographic locales where infectious diseases were noted to have emerged or resurged. Adapted from Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States, Department of Health and Human Services, Centers for Disease Control and Prevention, 1994. Reprinted from Figure 113-1 of Chapter 13, Introduction to Infectious Diseases: Host-Pathogen Interactions by Madoff, L.C. and Kasper, D.L. in Fauci, A.S., Kasper, D.L., Longo, D.L., Braunwald, E., Hauser, S.L., Jameson, J.L., and Losculzo, J. (Eds.), 2008. Harrison's Principles of Internal Medicine. McGraw Hill Medical, New York, p. 750, with permission.

Bioterrorism

While the threat of bioterrorism is small, all laboratories must be trained to recognize bioterrorism agents and to implement appropriate precautions along with notification of appropriate public health authorities. Agents of bioterrorism include *Bacillus anthracis, Francisella tularensis,* and *Yersinia pestis*. New technologies that are developed to rapidly detect common pathogens in direct specimens for the improvement of patient care can also be utilized to detect bioterrorism agents more quickly should the need arise, as long as the physician thinks of this unlikely possibility. Such methods may help to reduce the consequences of such an event.

Further Reading

Arya, M., et al., 2005. Basic principles of real-time quantitative PCR. Expert Rev. Mol. Diagn. 5, 209–219.

Ash, L.R., Orihel, T.C., 2007. Ash & Orihel's Atlas of Human Parasitology. American Society for Clinical Pathology, Hong Kong.

Bourbeau, P.P., Ledeboer, N.A., 2013. Automation in clinical microbiology. J. Clin. Microbiol. 51, 1658–1665.

Chan, J.F., et al., 2012. Is the discovery of the novel human betacoronavirus 2c EMC/2012 (HCoV-EMC) the beginning of another SARS-like pandemic? J. Infect. 65, 477–489.

Cohen, J.a.P., Powderly, W.G. (Eds.), 2004. Infectious Diseases. Mosby, New York.

Croxatto, A., et al., 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol. Rev. 36, 380–407.

Dunne Jr., W.M., et al., 2012. Next-generation and whole-genome sequencing in the diagnostic clinical microbiology laboratory. Eur. J. Clin. Microbiol. Infect. Dis. 31, 1719–1726.

Fauci, A.S., Kasper, D.L., Longo, D.L., Braunwald, E., Hauser, S.L., Jameson, J.L. et al., (Eds.), 2008. Harrison's Principles of Internal Medicine, McGraw Hill Medical, New York.

Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2007. Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier, St. Louis.

Garcia, L.S., 2009. Practical Guide to Diagnostic Parasitology. American Society for Microbiology, Washington, DC.

Greig, J.D., Lee, M.B., 2012. A review of nosocomial norovirus outbreaks: infection control interventions found effective. Epidemiol. Infect. 140, 1151–1160.

Karch, H., et al., 2012. The enemy within us: lessons from the 2011 European *Escherichia coli* O104:H4 outbreak. EMBO Mol. Med. 4, 841–848.

Knife, D.M., Howley, P.M., Cohen, J.I., Griffin, D.E., Lamb, R.A., Martin, M.A. et al., (Eds.), 2007. Field's Virology, Wolters Kluwer, Lippincott Williams & Wilkins, Philadelphia.

Larone, D.H., 2011. Medically Important Fungi: A Guide to Identification. American Society for Microbiology, Washington, DC.

Mahy, M., et al., 2009. Measuring the impact of the global response to the AIDS epidemic: challenges and future directions. J. Acquir. Immune Defic. Syndr. 52 (Suppl. 2), S152–S159.

Mellmann, A., et al., 2011. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. PLoS One 6, e22751.

Morens, D.M., Fauci, A.S., 2012. Emerging infectious diseases in 2012: 20 years after the institute of medicine report. MBio 3.

Murray, P.R., Rosenthal, K.S., Pfaller, M.A., 2013. Medical Microbiology. Elsevier Saunders, Philadelphia, PA.

Neely, L.A., et al., 2013. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci. Transl. Med. 5, 182ra54.

Nordmann, P., et al., 2011. The emerging NDM carbapenemases. Trends Microbiol. 19, 588–595.

Olsen, R.J., et al., 2012. Bacterial genomics in infectious disease and the clinical pathology laboratory. Arch. Pathol. Lab. Med. 136, 1414–1422.

Tascini, C., et al., 2012. The 2011 revolution in antimicrobial susceptibility testing and reporting: what the ICU physicians need to know. Minerva Anestesiol. 78, 88–93.

Versalovic, J., Carroll, K.C., Funke, G., Jorgensen, J.H., Landry, M.L., Warnock, D.W. (Eds.), 2011. Manual of Clinical Microbiology, ASM Press, Washington, DC.

Watanabe, Y., et al., 2012. The changing nature of avian influenza A virus (H5N1). Trends Microbiol. 20, 11–20.

Winn Jr., W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P., et al., 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Lippincott Williams & Wilkins, Philadelphia.

Wolk, D.M., et al., 2012. PCR-electrospray ionization mass spectrometry: the potential to change infectious disease diagnostics in clinical and public health laboratories. J. Mol. Diagn. 14, 295–304.

Relevant Websites

www.cdc.gov/ – Centers for Disease Control and Prevention
www.microbelibrary.org/ – American Society for Microbiology
www.microbeworld.org/ – American Society for Microbiology
www.microbiologyonline.org.uk/ – Society for General Microbiology, UK
www.who.int/en/ – World Health Organization