is detected by chemiluminescence. bDNA assays for viral load of HIV, hepatitis B virus, and hepatitis C virus have been approved by the FDA. The advantage of bDNA assays over PCR is that only a single heating/annealing step is required to hybridize the target-binding probe to the target sequence for amplification.

APPLICATIONS OF NUCLEIC ACID TESTS

The number of FDA-approved NAATS has increased dramatically and includes tests for *Mycobacterium tuberculosis*, *N. gonorrhoeae*, *C. trachomatis*, group B *Streptococcus*, and methicillin-resistant *S. aureus*. FDA-approved multiplex NAAT panels for detection of several respiratory or gastrointestinal pathogens also are available. Again, many laboratories have used commercially available reagents and analyte-specific reagents to create laboratory-developed tests for diagnostic use. Nucleic acid tests are useful to detect and identify difficult-to-grow or noncultivable bacterial pathogens such as *Legionella*, *Ehrlichia*, *Rickettsia*, *Babesia*, *Borrelia*, and *Tropheryma whipplei*. In addition, methods for rapid detection of agents of public health concern, such as *Franciscella tularensis*, *Bacillus anthracis*, smallpox virus, and *Yersinia pestis*, have been developed and are available in regional (state) laboratories and at the Centers for Disease Control and Prevention.

Nucleic acid tests are also used to determine how close the relationship is among different isolates of the same species of pathogen. The demonstration that bacteria of a single clone have infected multiple patients in the context of a possible source of transmission (e.g., a health care provider) offers confirmatory evidence for an outbreak. Pulsed-field gel electrophoresis remains the gold standard for bacterial strain analysis. This method involves the use of restriction enzymes that recognize rare sequences of nucleotides to digest bacterial DNA, resulting in large DNA fragments. These fragments are separated by gel electrophoresis with variable polarity of the electrophoretic current and then are visualized. Similar band patterns (i.e., differences in ≤3 bands) suggest that different bacterial isolates are closely related, or clonal. Simpler methods of strain typing include sequencing of single or multiple genes and PCR-based amplification of repetitive DNA sequences in the bacterial chromosome. Whole bacterial genome sequencing has been used in investigation of some outbreaks, but this method is still under development.

Future applications of nucleic acid testing probably will include the replacement of culture for identification of many pathogens with additional multiplex NAAT and solid-state DNA/RNA chip technology, in which thousands of unique nucleic acid sequences can be detected on a single silicon chip.

SUSCEPTIBILITY TESTING OF BACTERIA

A principal responsibility of the clinical microbiology laboratory is to determine which antimicrobial agents inhibit a specific bacterial isolate. Such testing is used for patient care and for monitoring of infection control problems, such as methicillin-resistant *S. aureus* or vancomycin-resistant *Enterococcus faecium*. Two approaches are useful. The first is a qualitative assessment of susceptibility, with responses categorized as susceptible, resistant, or intermediate. This approach can involve either the placement of paper disks containing antibiotics on an agar surface inoculated with the bacterial strain to be tested (Kirby-Bauer or disk-diffusion method), with measurement of the zones of growth inhibition after incubation, or the use of broth cultures containing a set concentration of antibiotic (breakpoint method). These methods have been calibrated carefully against quantitative methods and clinical experience with each antibiotic, and zones of inhibition and breakpoints have been determined on a species-by-species basis.

The second approach is to inoculate the test strain of bacteria into a series of cultures with increasing concentrations of antibiotic. The lowest concentration of antibiotic that inhibits visible microbial growth of the bacteria is known as the *minimal inhibitory concentration* (MIC). If tubes in which no growth is seen are subcultured, the minimal concentration of antibiotic required to kill 99.9% of the starting inoculum also can be determined (*minimal bactericidal concentration,* or MBC). The MIC value can be given a categorical interpretation of susceptible, resistant, or intermediate and so is more widely used than the MBC. Quantitative susceptibility testing using microbroth dilution in microwell plates or other miniaturized testing platforms has been automated and is used commonly in clinical laboratories. The epsilometer test (E-test), a novel method for determination of the MIC, uses a plastic strip with a known gradient of antibiotic concentrations along its length. When the strip is placed on the surface of an agar plate seeded with the bacterial strain to be tested, antibiotic diffuses into the medium, and bacterial growth is inhibited. For some organisms, such as obligate anaerobes and some β-hemolytic streptococci, routine susceptibility testing generally is not performed because of the difficulty of growing the organisms or the predictable sensitivity of most isolates to specific antibiotics.

SUSCEPTIBILITY TESTING OF FUNGI

With the advent of many new agents for treating yeasts and systemic fungal agents, the need for testing of individual isolates for susceptibility to specific antifungal agents has increased. In the past, few laboratories participated in such testing because of a lack of standard methods like those available for testing bacterial agents. However, several systems have been approved for antifungal susceptibility testing. These methods, which determine the minimal fungicidal concentration (MFC), are similar to the broth microdilution methods used to determine the MIC for bacteria. The E-test method is approved for testing the susceptibility of yeasts to fluconazole, itraconazole, and flucytosine, and disk diffusion can be used to test the susceptibility of *Candida* species to fluconazole and voriconazole. Methods for determining the MFC of a drug against fungal agents such as *Aspergillus* species are technically difficult, and most clinical laboratories refer requests for such testing to reference laboratories.

ANTIVIRAL TESTING

See Chaps. 214e and 215e.