Classification

Ellen Jo Baron

General Concepts

Classification

Bacteria are classified and identified to distinguish among strains and to group them by criteria of interest to microbiologists and other scientists.

Nomenclature

Bacteria are named so that investigators can define and discuss them without the necessity of listing their characteristics.

Species

Species, groups of similar organisms within a genus, are designated by biochemical and other phenotypic criteria and by DNA relatedness, which groups strains on the basis of their overall genetic similarity.

Diagnostic Identification

Bacteria are identified routinely by morphological and biochemical tests, supplemented as needed by specialized tests such as serotyping and antibiotic inhibition patterns. Newer molecular techniques permit species to be identified by their genetic sequences, sometimes directly from the clinical specimen.

Subtyping

Because of differences in pathogenicity or the necessity to characterize a disease outbreak, strains of medical interest are often classified below the species level by serotyping, enzyme typing, identification of toxins or other virulence factors, or characterization of plasmids, protein patterns, or nucleic acids.

New and Unusual Species

Laboratories have no difficulty identifying most bacteria. Problems develop with atypical strains and rare or newly described species; misidentification can lead to inappropriate patient care. Therefore, laboratory personnel and physicians (at least
infectious disease specialists) must remain current regarding changes in taxonomy and the recognition of new species.

**Role of the Clinical Laboratory**

Clinical laboratory scientists detect, isolate, identify, and determine the antimicrobial susceptibility patterns of clinically relevant microbes at the request of physicians, and interface with public health laboratories.

**INTRODUCTION**

Bacteria are classified and identified to distinguish one organism from another and to group similar organisms by criteria of interest to microbiologists or other scientists. The most important level of this type of classification is the species level.

A species name should mean the same thing to everyone. Within one species, strains and subgroups can differ by the disease they produce, their environmental habitat, and many other characteristics. Formerly, species were created on the basis of such criteria, which may be extremely important for clinical microbiologists and physicians but which are not a sufficient basis for establishing a species. Verification of existing species and creation of new species should involve biochemical and other phenotypic criteria as well as DNA relatedness. In numerical or phenetic approaches to classification, strains are grouped on the basis of a large number of phenotypic characteristics. DNA relatedness is used to group strains on the basis of overall genetic similarity.

Species are identified in the clinical laboratory by morphological traits and biochemical tests, some of which are supplemented by serologic assessments (e.g., identification of *Salmonella* and *Shigella* species). Because of differences in pathogenicity (*Escherichia coli*) or the necessity to characterize a disease outbreak (*Vibrio cholerae*, methicillin-resistant *Staphylococcus aureus*), strains of medical interest are often classified below the species level by serology or identification of toxins. Pathogenic or epidemic strains also can be classified by the presence of a specific plasmid, by their plasmid profile (the number and sizes of plasmids), or by bacteriophage susceptibility patterns (phage typing). Newer molecular biologic techniques have enabled scientists to identify some species and strains (without the use of biochemical tests) by identifying a specific gene or genetic sequence, sometimes directly from the clinical specimen.

Laboratories have no difficulty in identifying typical strains of common bacteria using commonly available test systems. Problems do arise, however, when atypical strains or rare or newly described species are not in the data base. Such difficulties are compounded when the strains are misidentified rather than unidentified, and so laboratory personnel and physicians (at least infectious diseases specialists) should be familiar with taxonomic reference texts and journals that publish papers on new species.
Bacterial nomenclature at the genus and species level changes often, based primarily on the use of newer genetic techniques. A species may acquire more than one name. In some cases the recognition of a new species results in a unique correlation with specific clinical problems. For example, recognition of *Porphyromonas gingivalis* as a unique species, separate from its previous inclusion within *Bacteroides melaninogenicus* (now known to be composed of several taxonomic groups of black-pigmenting anaerobic gram-negative bacilli), elucidated its role as a key pathogen in adult periodontitis. It is important to understand why these changes and synonyms exist in taxonomy.

The clinical laboratory is concerned with the rapid, sensitive, and accurate identification of microbes involved in producing disease. The number and types of tests done in such a laboratory depend on its size and the population it serves. Highly specialized or rarely performed tests should be done only by reference laboratories. Physicians, clinical laboratory personnel, and reference laboratory personnel must have a good working relationship if patients are to receive first-rate care.

In addition, the physician and the clinical laboratory personnel must know which diseases and isolates are reportable to public health laboratories and how to report them.

**Definitions**

**Taxonomy**

Taxonomy is the science of classification, identification, and nomenclature. For classification purposes, organisms are usually organized into subspecies, species, genera, families, and higher orders. For eukaryotes, the definition of the species usually stresses the ability of similar organisms to reproduce sexually with the formation of a zygote and to produce fertile offspring. However, bacteria do not undergo sexual reproduction in the eukaryotic sense. Other criteria are used for their classification.

**Classification**

Classification is the orderly arrangement of bacteria into groups. There is nothing inherently scientific about classification, and different groups of scientists may classify the same organisms differently. For example, clinical microbiologists are interested in the serotype, antimicrobial resistance pattern, and toxin and invasiveness factors in *Escherichia coli*, whereas geneticists are concerned with specific mutations and plasmids.

**Identification**

Identification is the practical use of classification criteria to distinguish certain organisms from others, to verify the authenticity or utility of a strain or a particular reaction, or to isolate and identify the organism that causes a disease.

**Nomenclature**

Nomenclature (naming) is the means by which the characteristics of a species are defined and communicated among microbiologists. A species name should mean the
same thing to all microbiologists, yet some definitions vary in different countries or microbiologic specialty groups. For example, the organism known as *Clostridium perfringens* in the United States is called *Clostridium welchii* in England.

**Species**

A bacterial species is a distinct organism with certain characteristic features, or a group of organisms that resemble one another closely in the most important features of their organization. In the past, unfortunately, there was little agreement about these criteria or about the number of features necessary to distinguish a species. Species were often defined solely by such criteria as host range, pathogenicity, or ability to produce gas during the fermentation of a given sugar. Without a universal consensus, criteria reflected the interests of the investigators who described a particular species. For example, bacteria that caused plant diseases were often defined by the plant from which they were isolated; also, each new *Salmonella* serotype that was discovered was given species status. These practices have been replaced by generally accepted genetic criteria that can be used to define species in all groups of bacteria.

**Approaches to Taxonomy**

**Numerical Approach**

In their studies on members of the family Enterobacteriaceae, Edwards and Ewing established the following principles to characterize, classify, and identify organisms (Lennette et al., 1985):

Classification and identification of an organism should be based on its overall morphologic and biochemical pattern. A single characteristic (pathogenicity, host range, or biochemical reaction), regardless of its importance, is not a sufficient basis for classifying or identifying an organism.

A large and diverse strain sample must be tested to determine accurately the biochemical characteristics used to distinguish a given species.

Atypical strains often are perfectly typical members of a given biogroup within an existing species, but sometimes they are typical members of an unrecognized new species.

In numerical taxonomy (also called computer or phenetic taxonomy) many (50 to 200) biochemical, morphological, and cultural characteristics, as well as susceptibilities to antibiotics and inorganic compounds, are used to determine the degree of similarity between organisms. In numerical studies, investigators often calculate the coefficient of similarity or percentage of similarity between strains (where strain indicates a single isolate from a specimen). A dendrogram or a similarity matrix is constructed that joins individual strains into groups and places one group with other groups on the basis of their percentage of similarity. In the dendrogram in Figure 3-1, group 1 represents three *Citrobacter freundii* strains that are about 95 percent similar and join with a fourth *C freundii* strain at the level of 90 percent similarity. Group 2 is composed of three *Citrobacter diversus* strains that are 95 percent similar, and group 3 contains two *E coli* strains that are 95 percent similar, as well as a third *E coli* strain to which they are 90
percent similar. Similarity between groups 1 and 2 occurs at the 70 percent level, and
group 3 is about 50 percent similar to groups 1 and 2.

In some cases, certain characteristics may be weighted more heavily; for example, the
presence of spores in Clostridium might be weighted more heavily than the organism's ability to use a specific carbon source. A given level of similarity can be equated with relatedness at the genus, species, and, sometimes, subspecies levels. For instance, strains of a given species may cluster at a 90% similarity level, species within a given genus may cluster at the 70 percent level, and different genera in the same family may cluster at the 50 percent or lower level (Fig. 3-1).

FIGURE 3-1 Example of dendrogram.

When this approach is the only basis for defining a species, it is difficult to know how many and which tests should be chosen; whether and how the tests should be weighted; and what level of similarity should be chosen to reflect relatedness at the genus and species levels.

Most bacteria have enough DNA to specify some 1,500 to 6,000 average-sized genes. Therefore, even a battery of 300 tests would assay only 5 to 20 percent of the genetic potential of a bacterium. Tests that are comparatively simple to conduct (such as those for carbohydrate utilization and for enzymes, presence of which can be assayed colorimetrically) are performed more often than tests for structural, reproductive, and regulatory genes, presence of which is difficult to assay. Thus, major differences may go undetected.

Other types of errors may occur when species are classified solely on the basis of phenotype. For example, different enzymes (specified by different genes) may catalyze
the same reaction. Also, even if a metabolic gene is functional, negative reactions can occur because of the inability of the substrate to enter the cell, because of a mutation in a regulatory gene, or by production of an inactive protein. There is not necessarily a one-to-one correlation between a reaction and the number of genes needed to carry out that reaction. For instance, six enzymatic steps may be involved in a given pathway. If an assay for the end product is performed, a positive reaction indicates the presence of all six enzymes, whereas a negative reaction can mean the absence or nonfunction of one to six enzymes. Several other strain characteristics can affect phenotypic characterization; these include growth rate, incubation temperature, salt requirement, and pH. Plasmids that carry metabolic genes can enable strains to carry out reactions atypical for strains of that species.

The same set of "definitive" reactions cannot be used to classify all groups of organisms, and there is no standard number of specific reactions that allows identification of a species. Organisms are identified on the basis of phenotype, but, from the taxonomic standpoint, definition of species solely on this basis is subject to error.

**Phylogenetic Approach**

The ideal means of identifying and classifying bacteria would be to compare each gene sequence in a given strain with the gene sequences for every known species. This cannot be done, but the total DNA of one organism can be compared with that of any other organism by a method called nucleic acid hybridization or DNA hybridization. This method can be used to measure the number of DNA sequences that any two organisms have in common and to estimate the percentage of divergence within DNA sequences that are related but not identical. DNA relatedness studies have been done for yeasts, viruses, bacteriophages, and many groups of bacteria.

Five factors can be used to determine DNA relatedness: genome size, guanine-plus-cytosine (G+C) content, DNA relatedness under conditions optimal for DNA reassociation, thermal stability of related DNA sequences, and DNA relatedness under conditions supraoptimal for DNA reassociation. Because it is not practical to conduct these genotypic or phylogenetic evaluations in clinical laboratories, the results of simpler tests usually must be correlated with known phylogenetic data. For example, yellow strains of *Enterobacter cloacae* were shown, by DNA relatedness, to form a separate species, *Enterobacter sakazakii*, but were not designated as such until results of practical tests were correlated with the DNA data to allow routine laboratories to identify the new species.

**Genome Size**

True bacterial DNAs have genome sizes (measured as molecular weight) between 1 X 10⁹ and 8 X 10⁹. Genome size determinations sometimes can distinguish between groups. They were used to distinguish *Legionella pneumophila* (the legionnaire's disease bacterium) from *Bartonella (Rickettsia) quintana*, the agent of trench fever. *L pneumophila* has a genome size of about 3 X 10⁹; that of *B quintana* is about 1 X 10⁹.

**Guanine-plus-Cytosine Content**
The G+C content in bacterial DNA ranges from about 25 to 75 percent. This percentage is specific, but not exclusive, for a species; two strains with a similar G+C content may or may not belong to the same species. If the G+C contents are very different, however, the strains cannot be members of the same species.

**DNA Relatedness under Conditions Optimal for DNA Reassociation**

DNA relatedness is determined by allowing single-stranded DNA from one strain to reassociate with single-stranded DNA from a second strain, to form a double-stranded DNA molecule (Figure 3-2). This is a specific, temperature-dependent reaction. The optimal temperature for DNA reassociation is 25 to 30°C below the temperature at which native double-stranded DNA denatures into single strands. Many studies indicate that a bacterial species is composed of strains that are 70 to 100 percent related. In contrast, relatedness between different species is 0 to about 65 percent. It is important to emphasize that the term "related" does not mean "identical" or "homologous." Similar but nonidentical nucleic acid sequences can reassociate.

**FIGURE 3-2 Diagram of DNA reassociation.** DNA is composed of two purine nucleoside bases, adenine (A) and guanine (G), and two pyrimidine nucleoside bases, thymine (T) and cytosine (C). Double-stranded DNA is formed through hydrogen bonds that can occur only between the complementary bases A and T or G and C. (Top) Perfectly reassociated DNA base sequence in which all nucleosides are paired by hydrogen bonds. (Middle) Perfectly paired DNA base sequence in the center with unpaired, single-strand ends on each strand. (Bottom) None of the bases in the sequence (left to right) GCTACGTCAGT on the top strand are complementary to the sequence TACGATGCAGT in the bottom strand.

**Thermal Stability of Related DNA Sequences**

Each 1 percent of unpaired nucleotide bases in a double-stranded DNA sequence causes a 1 percent decrease in the thermal stability of that DNA duplex. Therefore,
comparison between the thermal stability of a control double-stranded molecule (in which both strands of DNA are from the same organism) and that of a heteroduplex (DNA strands from two different organisms) allows assessment of divergence between related nucleotide sequences.

**DNA Relatedness under Supraoptimal Conditions for DNA Reassociation**

When the incubation temperature used for DNA reassociation is raised from 25-30°C below the denaturation temperature to only 10-15°C below the denaturation temperature, only very closely related (and therefore highly thermally stable) DNA sequences can reassociate. Strains from the same species are 60 percent or more related at these supraoptimal incubation temperatures.

**Defining Species on the Basis of DNA Relatedness**

Use of these five factors allows a species definition based on DNA. Thus, *E. coli* can be defined as a series of strains with a G+C content of 49 to 52 moles percent, a genome molecular weight of $2.3 \times 10^9$ to $3.0 \times 10^9$, relatedness of 70 percent or more at an optimal reassociation temperature with 0 to 4 percent divergence in related sequences, and relatedness of 60 percent or more at a supraoptimal reassociation temperature. Experience with more than 300 species has produced an arbitrary phylogenetic definition of a species to which most taxonomists subscribe: "strains with approximately 70% or greater DNA-DNA relatedness and with 5°C or less divergence in related sequences." When these two criteria are met, genome size and G+C content are always similar, and relatedness is almost always 60 percent or more at supraoptimal incubation temperatures. The 70 percent species relatedness rule has been ignored occasionally when the existing nomenclature is deeply ingrained, as is that for *E. coli* and the four *Shigella* species. Because these organisms are all 70 percent or more related, DNA studies indicate that they should be grouped into a single species, instead of the present five species in two genera. This change has not been made because of the presumed confusion that would result.

DNA relatedness provides one species definition that can be applied equally to all organisms. Moreover, it cannot be affected by phenotypic variation, mutations, or the presence or absence of metabolic or other plasmids. It measures overall relatedness, and these factors affect only a very small percentage of the total DNA.

**Polyphasic Approach**

In practice, the approach to bacterial taxonomy should be polyphasic (Fig. 3-3). The first step is phenotypic grouping of strains by morphological, biochemical and any other characteristics of interest. The phenotypic groups are then tested for DNA relatedness to determine whether the observed phenotypic homogeneity (or heterogeneity) is reflected by phylogenetic homogeneity or heterogeneity. The third and most important step is reexamination of the biochemical characteristics of the DNA relatedness groups. This allows determination of the biochemical borders of each group and determination of reactions of diagnostic value for the group. For identification of a given organism, the importance of specific tests is weighted on the basis of correlation with DNA results. Occasionally, the reactions commonly used will not distinguish completely between two
distinct DNA relatedness groups. In these cases, other biochemical tests of diagnostic value must be sought.

**FIGURE 3-3 Bacterial identification.**

**Phenotypic Characteristics Useful in Classification and Identification**

**Morphologic Characteristics**

Both wet-mounted and properly stained bacterial cell suspensions can yield a great deal of information. These simple tests can indicate the Gram reaction of the organism; whether it is acid-fast; its motility; the arrangement of its flagella; the presence of spores, capsules, and inclusion bodies; and, of course, its shape. This information often can allow identification of an organism to the genus level, or can minimize the possibility that it belongs to one or another group. Colony characteristics and pigmentation are also quite helpful. For example, colonies of several *Porphyromonas* species autofluoresce under long-wavelength ultraviolet light, and *Proteus* species swarm on appropriate media.

**Growth Characteristics**

A primary distinguishing characteristic is whether an organism grows aerobically, anaerobically, facultatively (i.e., in either the presence or absence of oxygen), or microaerobically (i.e., in the presence of a less than atmospheric partial pressure of oxygen). The proper atmospheric conditions are essential for isolating and identifying bacteria. Other important growth assessments include the incubation temperature, pH, nutrients required, and resistance to antibiotics. For example, one diarrheal disease agent, *Campylobacter jejuni*, grows well at 42°C in the presence of several antibiotics; another, *Y enterocolitica*, grows better than most other bacteria at 4°C. *Legionella*, *Haemophilus*, and some other pathogens require specific growth factors, whereas *E coli* and most other Enterobacteriaceae can grow on minimal media.
Antigens and Phage Susceptibility

Cell wall (O), flagellar (H), and capsular (K) antigens are used to aid in classifying certain organisms at the species level, to serotype strains of medically important species for epidemiologic purposes, or to identify serotypes of public health importance. Serotyping is also sometimes used to distinguish strains of exceptional virulence or public health importance, for example with *V. cholerae* (O1 is the pandemic strain) and *E. coli* (enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteropathogenic serotypes).

Phage typing (determining the susceptibility pattern of an isolate to a set of specific bacteriophages) has been used primarily as an aid in epidemiologic surveillance of diseases caused by *Staphylococcus aureus*, mycobacteria, *P. aeruginosa*, *V. cholerae*, and *S. typhi*. Susceptibility to bacteriocins has also been used as an epidemiologic strain marker. In most cases recently, phage and bacteriocin typing have been supplanted by molecular methods.

Biochemical Characteristics

Most bacteria are identified and classified largely on the basis of their reactions in a series of biochemical tests. Some tests are used routinely for many groups of bacteria (oxidase, nitrate reduction, amino acid degrading enzymes, fermentation or utilization of carbohydrates); others are restricted to a single family, genus, or species (coagulase test for staphylococci, pyrrolidonyl arylamidase test for Gram-positive cocci).

Both the number of tests needed and the actual tests used for identification vary from one group of organisms to another. Therefore, the lengths to which a laboratory should go in detecting and identifying organisms must be decided in each laboratory on the basis of its function, the type of population it serves, and its resources. Clinical laboratories today base the extent of their work on the clinical relevance of an isolate to the particular patient from which it originated, the public health significance of complete identification, and the overall cost-benefit analysis of their procedures. For example, the Centers for Disease Control and Prevention (CDC) reference laboratory uses at least 46 tests to identify members of the Enterobacteriaceae, whereas most clinical laboratories, using commercial identification kits or simple rapid tests, identify isolates with far fewer criteria.

Classification Below and Above the Species Level

Below the Species Level

Particularly for epidemiological purposes, clinical microbiologists must distinguish strains with particular traits from other strains in the same species. For example, serotype O157:H7 *E. coli* are identified in stool specimens because of their association with bloody diarrhea and subsequent hemolytic uremic syndrome.

Below the species level, strains are designated as groups or types on the basis of common serologic or biochemical reactions, phage or bacteriocin sensitivity, pathogenicity, or other characteristics. Many of these characteristics are already used and accepted: serotype, phage type, colicin type, biotype, bioserotype (a group of strains
from the same species with common biochemical and serologic characteristics that set them apart from other members of the species), and pathotype (e.g., toxigenic Clostridium difficile, invasive E coli, and toxigenic Corynebacterium diphtheriae).

Above the Species Level

In addition to species and subspecies designations, clinical microbiologists must be familiar with genera and families. A genus is a group of related species, and a family is a group of related genera.

An ideal genus would be composed of species with similar phenotypic and phylogenetic characteristics. Some phenotypically homogeneous genera approach this criterion (Citrobacter, Yersinia, and Serratia). More often, however, the phenotypic similarity is present, but the genetic relatedness is not. Bacillus, Clostridium, and Legionella are examples of accepted phenotypic genera in which genetic relatedness between species is not 50 to 65 percent, but 0 to 65 percent. When phenotypic and genetic similarity are not both present, phenotypic similarity generally should be given priority in establishing genera. Identification practices are simplified by having the most phenotypically similar species in the same genus. The primary consideration for a genus is that it contain biochemically similar species that are convenient or important to consider as a group separate from other groups of organisms.

The sequencing of ribosomal RNA (rRNA) genes, which have been highly conserved through evolution, allows phylogenetic comparisons to be made between species whose total DNAs are essentially unrelated. It also allows phylogenetic classification at the genus, family, and higher taxonomic levels. The rRNA sequence data are usually not used to designate genera or families unless supported by similarities in phenotypic tests.

Designation of New Species and Nomenclatural Changes

Species are named according to principles and rules of nomenclature set forth in the Bacteriological Code. Scientific names are taken from Latin or Greek. The correct name of a species or higher taxon is determined by three criteria: valid publication, legitimacy of the name with regard to the rules of nomenclature, and priority of publication (that is, it must be the first validly published name for the taxon).

To be published validly, a new species proposal must contain the species name, a description of the species, and the designation of a type strain for the species, and the name must be published in the International Journal for Systematic Bacteriology (IJSB). Once proposed, a name does not go through a formal process to be accepted officially; in fact, the opposite is true: a validly published name is assumed to be correct unless and until it is challenged officially. A challenge is initiated by publishing a request for an opinion (to the Judicial Commission of the International Association of Microbiological Societies) in the IJSB. This occurs only in cases in which the validity of a name is questioned with respect to compliance with the rules of the Bacteriological Code. A question of classification that is based on scientific data (for example, whether a species, on the basis of its biochemical or genetic characteristics, or both, should be placed in a new genus or an existing genus) is not settled by the Judicial Commission, but by the preference and usage of the scientific community. This is why there are pairs of names such as Providencia rettgeri/Proteus rettgeri, Moraxella
More than one name may thus exist for a single organism. This is not, however, restricted to bacterial nomenclature. Multiple names exist for many antibiotics and other drugs and enzymes.

A number of genera have been divided into additional genera and species have been moved to new or existing genera, such as *Arcobacter* (new genus for former members of *Campylobacter*) and *Burkholderia* species (formerly species of *Pseudomonas*). Two former *Campylobacter* species (*cinaedi* and *fennelliae*) have been moved to the existing genus *Helicobacter* in another example.

The best source of information for new species proposals and nomenclatural changes is the *IJSB*. In addition, the *Journal of Clinical Microbiology* often publishes descriptions of newly described microorganisms isolated from clinical sources. Information, including biochemical reactions and sources of isolation, about new organisms of clinical importance, disease outbreaks caused by newer species, and reviews of clinical significance of certain organisms may be found in the *Annals of Internal Medicine*, *Journal of Infectious Diseases*, *Clinical Microbiology Reviews*, and *Clinical Infectious Diseases*. The data provided in these publications supplement and update *Bergey's Manual of Systematic Bacteriology*, the definitive taxonomic reference text.

**Assessing Newly Described Bacteria**

Since 1974, the number of genera in the family Enterobacteriaceae has increased from 12 to 28 and the number of species from 42 to more than 140, some of which have not yet been named. Similar explosions have occurred in other genera. In 1974, five species were listed in the genus *Vibrio* and four in *Campylobacter*; the genus *Legionella* was unknown. Today, there are at least 25 species in *Vibrio*, 12 *Campylobacter* species, and more than 40 species in *Legionella*. The total numbers of genera and species continue to increase dramatically.

The clinical significance of the agent of legionnaire's disease was well known long before it was isolated, characterized, and classified as *Legionella pneumophila*. In most cases, little is known about the clinical significance of a new species at the time it is first described. Assessments of clinical significance begin after clinical laboratories adopt the procedures needed to detect and identify the species and accumulate a body of data.

In fact, the detection and even the identification of uncultivatable microbes from different environments are now possible using standard molecular methods. The agents of cat scratch disease (*Bartonella henselae*) and Whipple's disease (*Tropheryma whippelii*) were elucidated in this manner. *Bartonella henselae* has since been cultured from several body sites from numerous patients; *T whippelii* remains uncultivated.

New species will continue to be described. Many will be able to infect humans and cause disease, especially in those individuals who are immunocompromised, burned, postsurgical, geriatric, and suffering from acquired immunodeficiency syndrome (AIDS). With today's severely immunocompromised patients, often the beneficiaries of advanced medical interventions, the concept of "pathogen" holds little meaning. Any organism is capable of causing disease in such patients under the appropriate conditions.
**Role of the Clinical Laboratory**

Clinical laboratory scientists should be able to isolate, identify, and determine the antimicrobial susceptibility pattern of the vast majority of human disease agents so that physicians can initiate appropriate treatment as soon as possible, and the source and means of transmission of outbreaks can be ascertained to control the disease and prevent its recurrence. The need to identify clinically relevant microorganisms both quickly and cost-effectively presents a considerable challenge.

To be effective, the professional clinical laboratory staff must interact with the infectious diseases staff. Laboratory scientists should attend infectious disease rounds. They must keep abreast of new technology, equipment, and classification and should communicate this information to their medical colleagues. They should interpret, qualify, or explain laboratory reports. If a bacterial name is changed or a new species reported, the laboratory should provide background information, including a reference.

The clinical laboratory must be efficient. A concerted effort must be made to eliminate or minimize inappropriate and contaminated specimens and the performance of procedures with little or no clinical relevance. Standards for the selection, collection, and transport of specimens should be developed for both laboratory and nursing procedure manuals and reviewed periodically by a committee composed of medical, nursing, and laboratory staff. Ongoing dialogues and continuous communication with other health care workers concerning topics such as specimen collection, test selection, results interpretation, and new technology are essential to maintaining high quality microbiological services.

**Biochemical and Susceptibility Testing**

Most laboratories today use either commercially available miniaturized biochemical test systems or automated instruments for biochemical tests and for susceptibility testing.

The kits usually contain 10 to 20 tests. The test results are converted to numerical biochemical profiles that are identified by using a codebook or a computer. Carbon source utilization systems with up to 95 tests are also available. Most identification takes 4 to 24 hours. Biochemical and enzymatic test systems for which data bases have not been developed are used by some reference laboratories.

Automated instruments can be used to identify most Gram-negative fermenters, nonfermenters, and Gram-positive bacteria, but not for anaerobes. Antimicrobial susceptibility testing can be performed for some microorganisms with this equipment, with results expressed as approximate minimum inhibitory drug concentrations. Both tasks take 4 to 24 hours. If semiautomated instruments are used, some manipulation is done manually, and the cultures (in miniature cards or microdilution plates) are incubated outside of the instrument. The test containers are then read rapidly by the instrument, and the results are generated automatically. Instruments are also available for identification of bacteria by cell wall fatty acid profiles generated with gas-liquid chromatography (GLC), analysis of mycolic acids using high performance liquid chromatography (HPLC), and by protein-banding patterns generated by polyacrylamide gel electrophoresis (PAGE). Some other instruments designed to speed laboratory diagnosis of bacteria are those that detect (but do not identify) bacteria in blood.
cultures, usually faster than manual systems because of continuous monitoring. Also available are many rapid screening systems for detecting one or a series of specific bacteria, including certain streptococci, *N meningitidis*, salmonellae, *Chlamydia trachomatis*, and many others. These screening systems are based on fluorescent antibody, agglutination, or other rapid procedures.

It is important to inform physicians as soon as a presumptive identification of an etiologic agent is obtained so that appropriate therapy can be initiated as quickly as possible. Gram stain and colony morphology; acid-fast stains; and spot indole, oxidase, and other rapid enzymatic tests may allow presumptive identification of an isolate within minutes.

**Role of the Reference Laboratory**

Despite recent advances, the armamentarium of the clinical laboratory is far from complete. Few laboratories can or should conduct the specialized tests that are often essential to distinguish virulent from avirulent strains. Serotyping is done only for a few species, and phage typing only rarely. Few pathogenicity tests are performed. Not many laboratories can conduct comprehensive biochemical tests on strains that cannot be identified readily by commercially available biochemical systems. Even fewer laboratories are equipped to perform plasmid profiles, gene probes, or DNA hybridization. These and other specialized tests for the serologic or biochemical identification of some exotic bacteria, yeasts, molds, protozoans, and viruses are best done in regional reference laboratories. It is not cost-effective for smaller laboratories to store and control the quality of reagents and media for tests that are seldom run or quite complex. In addition, it is impossible to maintain proficiency when tests are performed rarely. Sensitive methods for the epidemiologic subtyping of isolates from disease outbreaks, such as electrophoretic enzyme typing, rRNA fingerprinting, whole-cell protein electrophoretic patterns, and restriction endonuclease analysis of whole-cell or plasmid DNA, are used only in reference laboratories and a few large medical centers.

Specific genetic probes are now available commercially for identifying virulence factors and many bacteria and viruses. Genetic probes are among the most common methods used for identification of *Mycobacterium tuberculosis* and *M avium* complex in the U.S. today. Probes for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are now being used directly on clinical specimens with excellent sensitivity and almost universal specificity with same-day results. Mycobacterial probes are also being evaluated for direct specimen testing.

**Interfacing with Public Health**
Laboratories

Hospital and local clinical laboratories interact with district, state, and federal public health laboratories in several important ways (Fig. 3-4). The clinical laboratories participate in quality control and proficiency testing programs that are conducted by federally regulated agencies. The government reference laboratories supply cultures and often reagents for use in quality control, and they conduct training programs for clinical laboratory personnel.

All types of laboratories should interact closely to provide diagnostic services and epidemic surveillance. The primary concern of the clinical laboratory is identifying infectious disease agents and studying nosocomial and local outbreaks of disease. When the situation warrants, the local laboratory may ask the state laboratory for help in identifying an unusual organism, discovering the cause or mode of transmission in a disease outbreak, or performing specialized tests not done routinely in clinical laboratories. Cultures should be pure and should be sent on appropriate media following appropriate procedures for transport of biohazardous materials. Pertinent information, including the type of specimen; patient name (or number), date of birth, and sex; clinical diagnosis, associated illness, date of onset, and present condition; specific agent suspected, and any other organisms isolated; relevant epidemiologic and clinical data; treatment of patient; previous laboratory results (biochemical or serologic tests); and necessary information about the submitting party must accompany each request.
These data allow the state laboratory to test the specimen properly and quickly, and they provide information about occurrences within the state. For example, a food-borne outbreak might extend to many parts of the state (or beyond its boundaries). The state laboratory can alert local physicians to the possibility of such outbreaks.

Another necessary interaction between local and state laboratories is the reporting of notifiable diseases by the local laboratory. The state laboratory makes available to local laboratories summaries of the incidence of these diseases. The state laboratories also submit the summaries to the CDC weekly (or, for some diseases, yearly), and national summaries are published weekly in the *Morbidity and Mortality Weekly Report*.

Interaction between the CDC and state and federal laboratories is very similar to that between local and state laboratories. The CDC provides quality control cultures and reagents to state laboratories, and serves as a national reference laboratory for diagnostic services and epidemiologic surveillance. Local laboratories, however, must initially send specimens to the local or state public health laboratory, which, when necessary, forwards them to the CDC. The CDC reports its results back to the state laboratory, which then reports to the local laboratory.

**Hazards of Clinical Laboratory Work**

Clinical laboratory personnel, including support and clerical employees, are subject to the risk of infection, chemical hazards, and, in some laboratories, radioactive contamination. Such risks can be prevented or minimized by a laboratory safety program.

**Radiation Hazards**

Personnel who work with radioactive materials should have taken a radioactivity safety course; they should wear radiation monitor badges and be aware of the methods for decontaminating hands, clothing, work surfaces, and equipment. They should wear gloves when working with radioactive compounds. When they work with high-level radiation, they should use a hood and stand behind a radiation shield. Preparative radioactive work should be done in a separate room with access only by personnel who are involved directly in the work.

**Chemical Hazards**

Chemicals can harm laboratory personnel through inhalation or skin absorption of volatile compounds; bodily contact with carcinogens, acids, bases, and other harmful chemicals; or introduction of poisonous or skin-damaging liquids into the mouth. Good laboratory practices require that volatile compounds be handled only under a hood, that hazardous chemicals never be pipetted by mouth, and that anyone working with skin-damaging chemicals wear gloves, eye guards, and other personal protective equipment as necessary. Workers should be familiar with the materials safety data sheets (MSDS) posted in an accessible place in every laboratory. These forms contain information about chemical hazards and procedures for decontamination should an accident occur.

**Biologic Hazards**
Microbiologic contamination is the greatest hazard in clinical microbiology laboratories. Laboratory infections are a danger not only to the clinical laboratory personnel but also to anyone else who enters the laboratory, including janitors, clerical and maintenance personnel, and visitors. The risk of infection is governed by the frequency and length of contact with the infectious agent, its virulence, the dose and route of administration, and the susceptibility of the host. The inherent hazard of any infectious agent is affected by factors such as the volume of infectious material used, handling of the material, effectiveness of safety containment equipment, and soundness of laboratory methods. Body fluids from patients, particularly those containing blood, are considered potentially infectious for blood-borne pathogens, and must be handled appropriately.

If possible, agents that are treated differently, such as viruses as opposed to bacteria, or \textit{M tuberculosis} in contrast to \textit{E coli}, should be handled in different laboratories or in different parts of the same laboratory. When the risk category of an agent is known, it should be handled in an area with appropriate containment. All specimens sent for microbiological studies and all organisms sent to the laboratory for identification should be assumed to be potentially infectious. A separate area should be set aside for the receipt of specimens. Personnel should be aware of the potential hazards of improperly packed, broken, or leaking packages and of the proper methods for their handling and decontamination.

To prevent infection, personnel should wear moisture-proof laboratory coats at all times, wash their hands before and after wearing gloves and at the conclusion of each potential exposure to etiologic agents, refrain from mouth pipetting, and not eat, drink, smoke, or apply cosmetics in the laboratory. Immunization may be appropriate for employees who are exposed often to certain infectious agents, including hepatitis B, yellow fever, rabies, polioviruses, meningococci, \textit{Y pestis}, \textit{S typhi}, and \textit{Francisella tularensis}. Universal precautions, body substance isolation, and other mandated practices involve the use of personal protective equipment and engineering controls to minimize laboratory scientists' exposure to blood-borne pathogens, even when the risk of infection is unknown.

**Biosafety Levels**

Infectious agents are assigned to a biosafety level from 1 to 4 on the basis of their virulence. The containment levels for organisms should correlate with the biosafety level assigned. Biosafety level 1 is for well-defined organisms not known to cause disease in healthy humans; it includes certain nonvirulent \textit{E coli} strains (such as K-12) and \textit{B subtilis}. Containment level 1 involves standard microbiologic practices, and safety equipment is not needed.

Biosafety level 2, the minimum level for clinical laboratories, is for moderate-risk agents associated with human disease. Containment level 2 includes limited access to the work area, decontamination of all infectious wastes, use of protective gloves, and a biologic safety cabinet for use in procedures that may create aerosols. Examples of biosafety level 2 agents include nematode, protozoan, trematode, and cestode human parasites; all human fungal pathogens except \textit{Coccidioides immitis}; all members of the Enterobacteriaceae except \textit{Y pestis}; \textit{Bacillus anthracis}; \textit{Clostridium tetani}; \textit{Corynebacterium diphtheriae}; \textit{Haemophilus} species; leptospires; legionellae;
mycobacteria other than *M tuberculosis*; pathogenic *Neisseria* species; staphylococci, streptococci, *Treponema pallidum*; *V cholerae*; and hepatitis and influenza viruses. Clinical specimens potentially containing some biosafety level 3 agents, such as *Brucella* spp., are usually handled using biosafety level 2 containment practices.

Biosafety level 3 is for agents that are associated with risk of serious or fatal aerosol infection. In containment level 3, laboratory access is controlled, special clothing is worn in the laboratory, and containment equipment is used for all work with the agent. *M tuberculosis*, *Coccidioides immitis*, *Coxiella burnetii*, and many of the arboviruses are biosafety 3 level agents. Containment level 3 usually is recommended for work with cultures of rickettsiae, brucellae, *Y pestis*, and a wide variety of viruses, including human immunodeficiency viruses.

Biosafety level 4 indicates dangerous and novel agents that cause diseases with high fatality rates. Maximum containment and decontamination procedures are used in containment level 4, which is found in only a few reference and research laboratories. Only a few viruses (including Lassa, Ebola, and Marburg viruses) are classified in biosafety level 4.

**REFERENCES**


Center for Infectious Diseases. Reference/Diagnostic Services. Centers for Disease Control and Prevention, Atlanta, Ga.


Richmond JY, McKinney RW (eds): Biosafety in Microbiological and Biomedical Laboratories. 3rd Ed. Centers for Disease Control and Prevention, Atlanta, Ga., and National Institutes of Health, Bethesda, Md., 1993
