CHAPTER 1

Introduction

Taxonomy

There are different methods for classifying or grouping microorganisms, for example based on genetic relatedness, on phenotypic features, on epidemiologic characteristics, or on clinical effects. In this book, the genetic relatedness is used for taxonomy in most circumstances. Five main categories are used: prions, viruses, bacteria, fungi, and parasites, and within each (except for prions), there are several different subcategories. The value of classifying and naming organisms is as follows.

• Names carry information about pathogenesis, epidemiology, and antimicrobial susceptibility.
• A systematic approach might assist in constructing a microbiological differential diagnosis for solving a clinical problem.

The classification of microorganisms causing disease in humans is shown in Table 28.1 of the Appendices. Classification of organisms is also shown for each chapter or section.

Largely as a result of advances in genetics and the consequent ability to better classify organisms, taxonomy and nomenclature are changing rapidly. The following websites offer the most up-to-date classifications and nomenclatures of microorganisms.

• Bacteria: www.bacterio.net/-alintro.html
• Fungi: Mycobank (www.mycobank.org) and Index Fungorum (www.indexfungorum.org/)
• Parasites: www.cdc.gov/dpdx
**purposes of the clinical microbiology laboratory**

The purpose of the clinical microbiology laboratory is the detection and identification of microorganisms, susceptibility testing of isolated organisms to antimicrobial agents and, in some circumstances, the quantification of the number of organisms in body fluids.

**Principles of diagnostic testing**

Diagnostic testing can be used for clinical purposes (patient management), epidemiologic purposes (recognition of disease patterns, including trends and outbreaks), and for research. The following discussion applies primarily to testing for clinical purposes.

A diagnostic test should be considered when its results may help in deciding about a patient’s management. A patient’s clinical features may be so suggestive of the diagnosis, and the withholding of treatment may be so deleterious, that you would give therapy without any further ado (or diagnostic testing). For this patient, your belief in the probability of the diagnosis is above a threshold, which is called the **test-treat threshold** (Fig. 1.1).

Another patient’s clinical features may not be highly suggestive of the diagnosis, and withholding therapy may not carry a significant penalty. In this case, your belief in the probability of the diagnosis is so low that you think that neither testing nor treatment is appropriate. The probability of the diagnosis is below a threshold called the **no test-test threshold** (see Fig. 1.1).

Therefore deciding about diagnostic testing requires an appreciation of the following probabilities.

- The probability (what you believe to be the probability) of the diagnosis before the test is performed. This is called the **pretest probability**.

![Decision thresholds](image-url)
• The probability of the diagnosis above which you would treat the patient, irrespective of the results of a diagnostic test (test-treat threshold).
• The probability of the diagnosis below which you would not treat, irrespective of a test result (no test-test threshold).

Thus there are three zones of probability regarding treating and testing.
• Probability below the no treat-test threshold: NO ACTION.
• Probability between the two thresholds: TEST.
• Probability above the test-treat threshold: TREAT.

How do we know what the probabilities should be for these thresholds? These are determined by the benefits of treatment of patients with the disease (diagnosis), and the harm inflicted by treatment of the non-diseased as well as the diseased, and the harm inflicted by the test itself.

Each test has parameters of performance. For many blood tests, these are known, for example, as determined by the manufacturer or developer of the test. For some, these parameters are not really known, especially imaging tests.

Clinicians are interested in parameters called sensitivity and specificity. These are demonstrated in Table 1.1. This table is commonly used, and readers should become very familiar with it. The columns indicate the TRUE state of the patients (disease or no disease); the rows indicate the test results (test positive or negative).

**Sensitivity** means:
• the proportion of patients who really have the disease who have a positive test \((a/a + c)\); this is also called the true-positive rate (TPR)
• \((c/a + c)\) is the proportion of patients who really have the disease but who have a negative test; this is the false-negative rate, and is \((1 – sensitivity)\).

**Specificity** means:
• the proportion of patients who really do not have the disease who have a negative test \((d/b + d)\); this is also called the true-negative rate (TNR)
• \((b/b + d)\) is the proportion of patients who really do not have the disease, but who have a positive test. This is also called the false-positive rate, and is \((1 – specificity)\).

In clinical medicine, the question of interest is often as follows: If the test is positive, what is the probability of the patient having disease or, if the test is negative, what is the probability that the patient does not have disease? These are the predictive values.

<table>
<thead>
<tr>
<th>Disease</th>
<th>No disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test positive</td>
<td>a</td>
</tr>
<tr>
<td>Test negative</td>
<td>c</td>
</tr>
<tr>
<td>a + c</td>
<td>b + d</td>
</tr>
</tbody>
</table>

\(a = \text{true positives}; \) these are the cases in which the patient HAS the disease AND the test is POSITIVE.
\(b = \text{false positives}; \) these are the cases in which the patient DOES NOT have the disease BUT the test is POSITIVE.
\(c = \text{false negatives}; \) these are the cases in which the patient HAS the disease BUT the test is NEGATIVE.
\(d = \text{true negatives}; \) these are the cases in which the patient DOES NOT have the disease AND the test is NEGATIVE.
• The positive predictive value, i.e. the probability of disease if the test is positive, is \(a/a + b\).
• The negative predictive value, i.e. the probability of no disease if the test is negative, is \(d/c + d\).
These are determined not only by the sensitivity and specificity of the test, but also by the prevalence of the disease in the population from which the patient is drawn, or the pretest probability.

**Sensitivity**

Let us start with an example.

**Example 1**
You want to establish a test for screening blood donors for a viral infection. The donors are asymptomatic for the infection. You want to eliminate, to the best of your ability, any chance that infected blood could enter your donor pool, even if it means rejecting blood that actually might be fine. Therefore you want a very sensitive test. Such a test should detect everyone who has the infection (even if it means calling someone infected if they are not really infected). This means you want to minimize the number of false negatives. Conversely, the true-positive rate (sensitivity) is very high.

If the test is negative, there is no disease. A sensitive test is used to "rule out" a disease. Sensitivity is to rule OUT (SNOUT) (Fig. 1.2).

**Specificity**

**Example 2**
You want a test to test for an illness, for example a cancer, for which therapy is very toxic. You do not want to be giving toxic therapy to someone who does not really have the disease. Thus you want to eliminate, to the best of your ability, any chance of making the diagnosis of this disease in someone who does not really have the disease (false positives). That means you want a test with a very high true-negative rate (specificity).

If the test is positive, there is disease. A specific test is used to "rule in" a disease. Specificity is to rule IN (SPIN) (Fig. 1.3).
Diagnostic testing is like fishing with a net.

**Example 3**
Scenario: You want to catch large fish (3–5 cm across). If you use a net with small holes (2 cm across), you will catch all the large fish. However, you will also catch small fish that you do not want. This is analogous to using a sensitive test that is not specific. You will catch all the cases that you want (the large fish), but you will also catch cases that you do not want (the small fish) (Fig. 1.4).

On the other hand, if you use a net with larger holes (4 cm), you will not catch any small fish, and you will catch most large fish, but you will also miss some of the large fish that you do want. This is analogous to using a specific test that is not sensitive (Fig. 1.5).

There is always a tension between the sensitivity and the specificity of tests. As the sensitivity increases, the specificity decreases, and vice versa (Fig. 1.6).
How do we know the true state (disease or no disease)?

As can be seen in Table 1.1, determining the sensitivity and specificity of a test depends on knowing the patient’s true state, that is, is disease present or not? The method by which the true state is determined is often referred to as "the gold standard." This is the method which is often “accepted” as the definitive way to make the diagnosis. Because the parameters of a new test are dependent on the gold standard, the dependability of the gold standard is of the utmost importance. Unfortunately, attainment of a suitable gold standard may be difficult, and there are several potential pitfalls in studies of diagnostic tests in which a suitable gold standard is not used.

In the microbiology laboratory, the gold standard has, for many years and in many circumstances, been culture of the microorganism. The disadvantages of this are the following.

- An organism may grow poorly in culture, or not at all, e.g. Treponema pallidum, the cause of syphilis. (There may be many organisms that are unknown because they cannot be cultured in artificial media.) This reduces the sensitivity of culture.
Although an organism may be cultivable, culture may take many days or weeks, which might not be practicable for clinical medicine, e.g. *Mycobacterium tuberculosis*.

Because specimens are taken from sites that might harbor organisms other than the pathogen of interest, culture might detect an organism that is a “contaminant.” This reduces the specificity of culture. Therefore, in many circumstances, molecular tests have become the gold standard (see Chapter 2).
Microbiologic tests can be narrow spectrum, i.e. specific for a single organism (e.g. polymerase chain reaction, serologic tests, antigen detection tests), broad spectrum (e.g. culture in medium supporting the growth of many different organisms), or intermediate in spectrum, i.e. able to detect a limited number of organisms (e.g. blood smear).

When considering microbiologic testing, the following should be borne in mind.

• The general medical differential diagnosis.
• The microbial differential diagnosis.
• How knowing whether there is an organism and what it is will help in patient management (for therapy, for withholding therapy, or for public health measures such as isolation of the patient).
• To what level of specificity (i.e. genus, species, serotype, strain) an organism’s identification should be made.

**IMPORTANT:** to make a microbiologic diagnosis, you need specimens appropriate for microbiologic testing.

**Antimicrobial resistance**

The ability of pathogenic microorganisms to resist the effects of antimicrobial agents, antimicrobial resistance, is a very important and challenging problem in clinical medicine. Although the molecular mechanisms vary according to the different categories of organism (discussed separately within each category), the basic principles are the same. The measure of susceptibility of an organism is determined, generally, by allowing the organism to grow, in culture, in a medium containing varying concentrations.
of the antimicrobial agent. The lower the concentration that inhibits the growth of the
organism, the more susceptible the organism is to that agent. For bacteria and fungi, the
measure used is the minimal inhibitory concentration (MIC), while in viruses and par-
asites the measure usually used is the inhibitory concentration \( I_{50} \) (ID \(_{50} \)), the concentration
that causes 50% inhibition of growth (see Chapter 2 on laboratory methods). There are
conceptually two types of resistance: microbiologic resistance, meaning that the organism
is more resistant than other members of its species; and clinical resistance, meaning that
the organism is resistant to concentrations of the drug that can be safely achieved in the
infected tissue.

Resistance is, ultimately, determined by the genetic attributes of the organism. Some
organisms are inherently resistant and, to our knowledge, have always been resistant
to certain agents. This is sometimes called “native resistance.” Other organisms have
acquired resistance over time since the antimicrobial agent has been in existence (prior
to its existence, one could not have demonstrated susceptibility or resistance). The ability
to acquire resistance depends on the organism undergoing a genetic change. This can
occur by mutation or acquisition of new genetic material (discussed in the section on
antibacterial resistance in Chapter 9). The frequency of mutations varies among different
organisms. However, because microorganisms generally have very short generation
times compared with that of their hosts, mutations can occur relatively frequently.

Once an organism has become resistant to an antimicrobial agent, it can become
prevalent within a population of organisms by two processes:

- Darwinian selection: in circumstances in which the relevant antimicrobial agent is
  present in the organism’s environment, the susceptible organisms are inhibited or
  killed, while the resistant ones multiply and thrive, and eventually become the
  predominant or only population (Fig. 1.7).

![Fig. 1.7](image_url) How exposure to an antibiotic results in the resistant organisms becoming the dominant
organisms and then the only organisms.
• Resistant organisms spread to new areas: this occurs via the same routes by which susceptible organisms spread, e.g. by personal contact, by droplets, by the airborne route, or by arthropod vectors. In hospitals, where there is a high prevalence of resistant organisms, the hands of healthcare workers are an important mode of spread.

Further reading


