8.
Diagnostic Technologies: Selected Tropical Diseases

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Diagnostic Technologies; Selected Tropical Diseases

INTRODUCTION

Diagnosis has three important functions. One is to determine the nature of the individual's disease for the purpose of deciding on an appropriate course of treatment. A second function is to determine the prevalence of specific disease-producing organisms or agents in populations, to allow assessments of the impact of public health interventions. The third is to find out about the range of diseases affecting a population, the immune stages of populations, etc., for the purposes of research.

Diagnosis of tropical diseases is a challenge for the clinician or epidemiologist, because many of the diseases mimic a wide variety of infectious processes by presenting similar or ambiguous symptoms. In countries where these diseases are endemic, symptomatic ambiguity can also result from the common occurrence of multiple infections in a single individual. The proper identification of the species of the organism that is causing disease can be critical in clinical management, especially in serious illness.

Although each tropical disease can be diagnosed by a number of different methods, diagnostic techniques vary in their usefulness under different conditions. There are several parameters to consider in evaluating the usefulness of a diagnostic test: sensitivity, specificity, predictive value, cross-reactivity, and precision (388).

- Sensitivity is the ability of a test procedure to find a disease-producing agent or disease when it is present. A very sensitive test correctly identifies all infected individuals. Negative tests for individuals who have the disease are "false negatives. "
- ***Specificity*** is the ability of a test procedure to correctly determine that a disease-producing agent or disease is not present. A very specific test will correctly identify all uninfected individuals. Positive tests for individuals who do not have the disease are "false positives."
- Predictive ***value*** is an interaction of sensitivity, specificity, and the prevalence of the infection in the population being tested. The sensitivity and specificity of a test will give different numbers of false negative and false positive results depending on the prevalence of the infection in the population tested. If a test is conducted in a group of patients strongly suspected of having the disease, very few false positives would be expected, simply because very few people tested will actually be negative. Thus, positive results will be readily accepted as true. Conversely, if the same test is conducted in a population in which very few people are thought to have the disease, the positives would have a higher probability of being false positives, and in fact, false positives may outnumber true positives. The negative test results will mostly be true negatives.
- ***Cross-reactivity*** is an aspect of immunodiagnostic tests related to, yet different from, specificity. A cross-reaction occurs when the test correctly identifies the appropriate chemical entity, but that chemical happens to exist in a different organism than the one tested for. A false positive results.
- ***Precision*** is reproducibility, the ability of a test procedure to give consistent results in repeated trials of the same sample.

Broadly generalizing, there are two categories of conventional diagnostic technologies:

1. Direct examination of blood, stool, urine, sputum, tissue biopsy, or cultured isolates

using simple equipment (e.g., a light microscope) and reagents.

2. Serologic examination to detect antibodies to the pathogen or to detect the pathogen or its byproducts (antigen) in a sample of the patient's blood. Serologic methods have important advantages over direct examination, but require in most cases specialized equipment and reagents and have never achieved wide practical use.

Biotechnology is leading to the development of new diagnostic methods based on the use of monoclinal antibodies (MAbs) and nucleic acid (DNA or RNA) hybridization and promises to revolutionize diagnosis by offering quick, simple, accurate tests when and where they are needed.

The conventional methods of diagnosing infectious diseases and identifying disease-producing organisms include the following:

- the clinical impression of the physician or other health worker (this is the most widely used method of diagnosis);
- direct examination of clinical specimens (e.g., blood, stool, urine, sputum, or tissue biopsy) using light or electron microscopy to identify the disease-producing organism (e.g., malaria parasites, intestinal amebae and helminth eggs, leishmanial organisms, mycobacteria that cause tuberculosis and leprosy, filaria);
- X-ray or computed tomography (CT) scan to image internal pathogens (e.g., amebic abscesses, echinococcal cysts); occasionally useful, but not routine;
- xenodiagnosis, by permitting an insect vector to feed on a patient and then examining the insect by light microscopy to look for the disease-producing organism after it has multiplied to a detectable density (e.g., to diagnose Chagas' disease);
- culture of a specimen from a patient in a test medium, tissue culture, or by inoculation into an animal to allow it to multiply to a detectable density, followed by direct examination for the disease-producing organism, or use of serologic techniques (e.g., for many bacteria and viruses);
- skin tests, demonstrating hypersensitivity reactions to disease antigen (e.g., to diagnose tuberculosis);
- serologic methods to detect antibodies to the pathogenic organism in the patient's serum; and
- demonstration of a pathogen byproduct, such as an antigen by chemical methods (e.g., schistosomes).

Photo credit: Office of Technology Assessment

Xenodiagnosis, by allowing laboratory-raised reduviid bugs to feed on suspected Chagas' disease victim.

CONVENTIONAL DIAGNOSTIC TECHNIQUES

Direct Examination

Laboratory diagnostic methods that directly identify a disease-producing organism by microscopy of clinical samples can provide a definitive diagnosis. Constraints on this approach include the following:

- specially trained personnel are required;
- the work quickly becomes boring and repetitious, while still demanding concentration and attention to detail;
- procedures are relatively time-consuming and require equipment and materials that are often inadequate in endemic countries;
- in some cases, the organisms are not detectable either because:
  —they are not present in routine clinical samples (e.g., the organisms that cause trichinosis, toxoplasmosis, hydatid disease, hepatic capillariasis, toxocariasis);
  —they are not detectable in routine clinical samples until a later stage of infection (e.g., filaria); or
  —their densities are very low, making sensitive diagnosis difficult (e. g., in chronic malaria and chronic Chagas' disease); and
- identifying the disease-producing organism is difficult because some species appear similar (e.g., intestinal amebae, malarial parasites, the African schistosomes with terminally spined eggs, leishmanial organisms).

Serologic Diagnostic Techniques

Serologic diagnosis depends on two types of immunologic methods, which are discussed further below:

- methods for detecting specific antibodies (the immunoglobulins IgG, IgM, IgA, IgE, or IgD) in a sample of the patient's blood; and
- methods involving the use of immunoglobulins for the detection of antigen (the disease-producing organism or its byproducts) in a sample of the patient's blood.

For the detection of antibodies, there are a variety of immunologic methods, all of which can be adjusted by dilution of reagents to give a measure of intensity of reaction (a titer). Because the various immunoglobulins have different roles and develop at different times in an infection, different titers will be obtained at various stages in the infection. (For instance, the immunoglobulin IgM is usually an early but short-lived immune response to infection, whereas IgG production develops more slowly and then continues long after resolution of the infection.) A positive test for antibodies may indicate current infection or the immunity that follows exposure to the infectious agent. In some cases, the test may remain positive for extended periods after the infectious agent has been eliminated by drug treatment.

For the detection of antigen, methods have not been widely developed until recently, for technical reasons. The current advances in MAb technology make antigen detection more feasible.

Serologic Methods for Detection of Antibody

The principal serologic methods for detecting antibodies in a patient's blood are described below,

1. Complement fixation (CF) test. This method is well established and has been applied to all viral, bacterial, and parasitic diseases. Complement, a substance normally present in the blood, fixes or binds antigen to antibody. The CF test is performed by adding the known antigen and complement (prepared in the laboratory) to an individual's serum in a test tube. If antibodies are present in the patient's serum, antigen-antibody complexes are formed. No reaction indicates a lack of specific antibody. The CF test requires well-standardized reagents, some of which have limited shelf-life or limited availability in the tropics.

The CF test is widely used for viral diagnosis and is most effective for parasite diagnosis of American trypanosomiasis (Chagas' disease) and

schistosomiasis. It is highly specific, but relatively insensitive. It requires large amounts of antigen per test. The CF testis performed adequately only at specialized centers, and it is not considered a test for general, practical use in the tropics (388).

2. Agglutination assays. Clumping of particles due to the interaction of antibody and antigen is the basis of all agglutination tests. In most variations, the disease-producing organism or its by-product (antigen) is fixed to particles, and the particles are added to a test sample that may contain the complementary antibody. If the complementary antibody is present, the particles agglutinate. Each variation of this technique has acquired its own name. The principal agglutination techniques for detection of antibody are described below. (The complementary techniques for detection of antigen are noted in a later section.)

a. Direct agglutination test. In the direct agglutination test, whole organisms suspected of causing disease are added to the individual's serum. If antibody is present in the serum, the organisms clump together. The test is simple, but it requires pure, stable antigen preparations (from culture in vivo or in vitro), the test result interpretation is subjective, and there can be complicating auto-agglutination reactions that result in false positives.

b. Indirect hemagglutination (IHA) test. In IHA tests, the antigen is attached to a carrier, such as specially prepared red blood cells or latex particles. When the individual's serum is added, the particles clump if specific antibody is present. II-1A tests have been developed for malaria, Chagas' disease, leishmaniasis, amebiasis, rotavirus, and hydatid disease. IHA tests are simple to perform, can be used to test minute volumes of serum, and can be automated but the end-point reading is subjective. The preparation of test particles is not reproducible, the antigens have short shelf-lives, and there are problems in standardizing antigens from batch to batch.

c. Hemagglutination inhibition (HI) test. Various micro-organisms can clump or agglutinate red blood cells from test animals under specified conditions. In the HI test, prepared red blood cells, an appropriate micro-organism (e.g., a virus), and a test serum are combined. If the individual's serum contains antibody specific to that virus, the hemagglutination will be inhibited, because the antibody combines with the organism and cannot react with the red blood cells. The lack of agglutination indicates a positive test.

3. Neutralization test. Many viruses damage cultured cells in certain detectable ways. When test serum containing a specific antibody is added to a test well containing the cell culture and the virus (both known quantities prepared in the laboratory), the damage is inhibited, indicating a positive test for the antibody. Neutralizing antibody is usually the first to appear, early in the acute phase of the infection.

4. Precipitin (or immunodiffusion) test. With this method, two separate wells are cut into a semisolid substrate such as agar. Serum components are put in one well and antigen in the other. As they migrate, an observable precipitation line is formed where antibody and antigen combine. No precipitation line forms in the absence of antibody.

Counterimmunoelectrophoresis (CIE) and immunoelectrophoresis are adaptations of the precipitin test in which an electric current is passed through the substrate, causing the reactive components to migrate more rapidly and with greater resolution.

The relatively simple diffusion methods are suited to the tropics, but these methods are of limited practical value because of their insensitivity and the long period of time before results. The electrophoretic methods require equipment and a power supply, and the results are complex and somewhat difficult to interpret (98).

5. Labeled immunodiagnostic reagent assays. The various types of labeled immunodiagnostic reagent assays all involve a similar procedure. The test antigen is attached to a slide or test well (e.g., blood drops from malaria-parasitized monkeys are dried on microscope slides; in vitro cultured organisms are fixed to plastic test wells). The patients' serum is incubated as a drop on the slide or in the well. If antibody is in the serum, it binds to the fixed antigen. The excess serum is washed off, leaving the antibody sticking to the antigen on the slide. Then a second antibody is added that reacts with any antibody-antigen complexes that

were formed. This second reaction depends on the unusual structure of antibodies—one end of an antibody is a totally unique fit for just one antigen, and the other end is a totally generic molecule. The second lab-prepared antibody has a unique end which recognizes the generic end of other antibodies. This second antibody is also linked to a chemical marker that is detectable—a fluorescent molecule, a radioactive molecule, or an enzyme.

One type of labeled immunodiagnostic assay, the indirect fluorescent antibody (IFA) test, uses slides prepared with antigen attached (usually a fixed, cultured organism). After serum antibody binds to the antigen, the second lab-prepared antibody is added to react with any antibody-antigen complexes that were formed. This second antibody is linked to a chemical marker that fluoresces under ultraviolet light. A special microscope is used to detect fluorescence. If the slide fluoresces, it indicates that the original human serum contained the antibodies in question. Microscope slides are easily prepared with antigens stable for long periods for a number of viruses, bacteria, and parasites. Disadvantages of IFA tests are that a well-maintained, carefully calibrated, and expensive fluorescent microscope is needed, the test is time-consuming and somewhat subjective, and with many helminths, there are cross-reactions (388,421)

The enzyme-linked immunosorbent assay (ELISA) is complex to describe, yet simple and elegant in its determination. It is rapidly being adapted to the diagnosis of a wide range of organisms. As shown in figure 8-1, the ELISA for the detection of antibody involves attaching specific antigen (a known quantity of test organisms prepared in the laboratory) to a test well or plate, then adding the patient's serum with suspected specific antibody, then adding a lab-prepared second antibody which is linked to an enzyme. The final component added is a chemical substrate whose color changes by the action of the enzyme. The degree of color change is read qualitatively by eye or quantitatively by photometric instrument to give an indication of the amount of antibody present in the serum specimen.

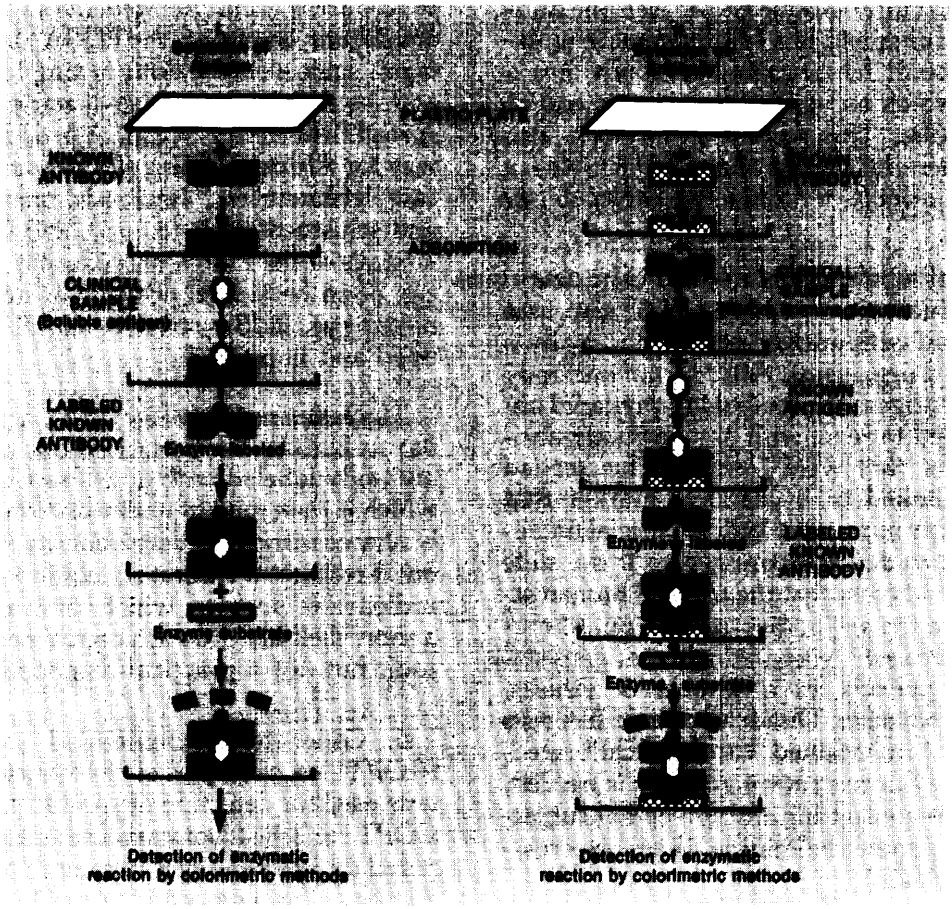
The ELISA uses small sample volumes. Large numbers of specimens can be processed, so the procedure is useful for epidemiologic studies. For developing countries, the ELISA has clear advantages, since it can be done in simple laboratories, and the reagents are stable if refrigerated. Widespread field testing of ELISA procedures is under way for African sleeping sickness and Chagas' disease, leishmaniasis, amebiasis, malaria, filariasis, and schistosomiasis (82,353,389) One great advantage of the ELISA procedure is that the result can often be judged positive or negative by the naked eye. ELISA tests are rapidly being developed and improved.

The radioimmunoassay (RIA), a procedure similar to ELISA, can be carried out using a second antibody labeled with a radioactive compound, which is then read in a scintillation' counter. RIA is very sensitive and very quantitatively accurate, but it requires a laboratory equipped to deal with radioactive isotopes, which carry a risk and have a short shelf-life. RIA has been a valuable research tool, but not a practical diagnostic tool (335).

6. Other tests: circumoval precipitin tests (COPT) are examples of the variety of serologic tests used for detection of specific pathogens. The COPT for schistosomiasis uses standardized prepared schistosome eggs obtained from animal infections. The eggs are incubated in patient sera, which causes a characteristic precipitate to form around the egg, if antischistosomal antibodies are present.

The thin-layer immunoassay for the detection of antibody is depicted in figure 8-2. This technique uses antigen in test plates to capture specific antibody from test serum. The antigen-antibody complexes form a thin layer that attracts water. When the test plate is exposed to water vapor, water visibly condenses on the immune complexes in large droplets which form a pattern distinct from the background. The thin-layer immunoassay has been used to detect the presence of viruses, schistosomes, and amebae. It requires relatively large amounts of antigen, however, and in some cases has low sensitivity (421).

Figure 8-1 .—Enzyme=Linked Immunosorbent Assay (ELISA) Technique for Detecting Antigen or Antibody



1. Detection of antigen in a clinical sample:
- **Antibody to the antigen being tested for is adsorbed to a plastic plate.**
- **The clinical sample is added. If the specific antigen is present, antigen-antibody complex forms on the plastic plate.**
- **A second antibody to the antigen, with a specific enzyme attached, is added to the plate. The enzyme-labeled antibody adheres where antigen-antibody complexes were formed.**
- **An enzyme substrate is added. If it comes in contact with the enzyme, a reaction occurs that produces a visible color change, If no complexes are formed, there is no reaction.**

II. Detection of antibody in a clinical sample:
- **The process is the same as the one for detecting antigen, but includes one additional step: known antigen is added, resulting in one more layer in the final complex.**

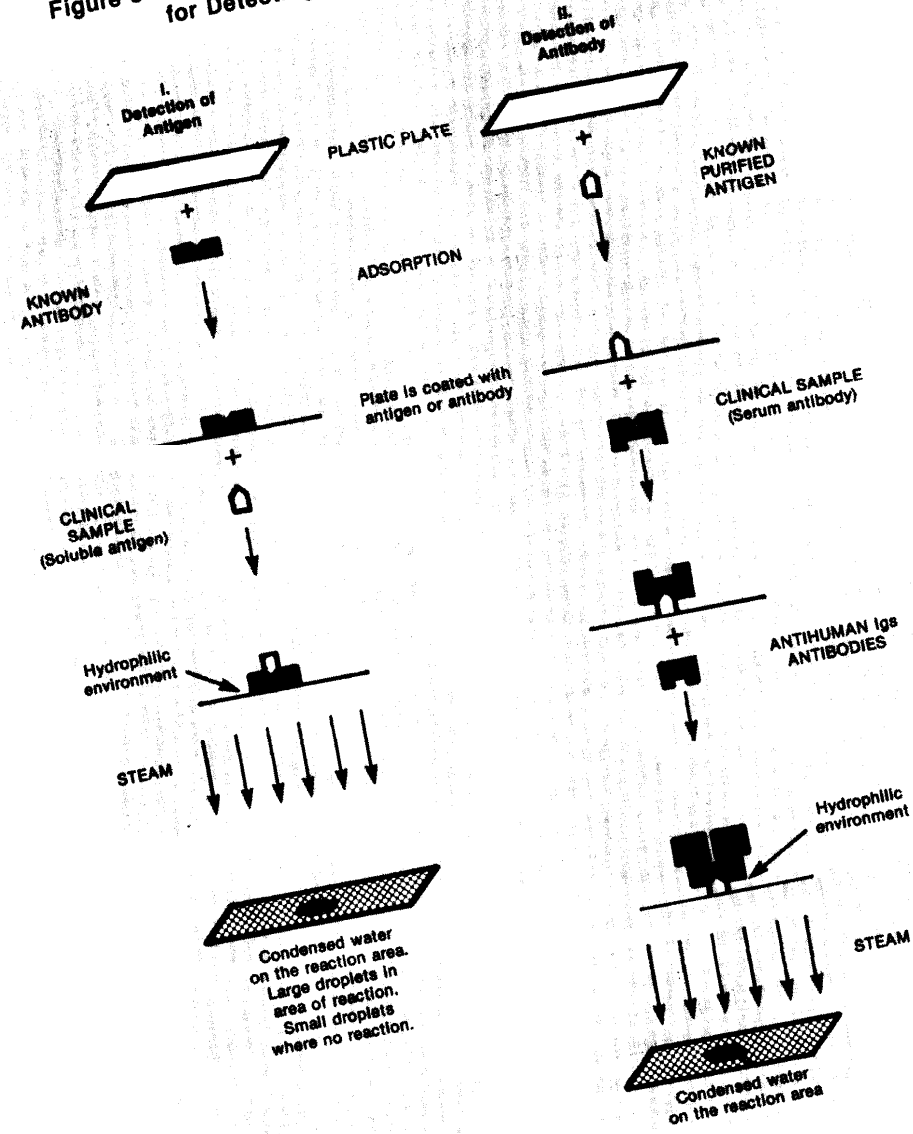
SOURCE: World Health Organization, *Rapid Laboratory Techniques for the Diagnosis of Viral Infections,* **Technical Report Series #661 (Geneva: WHO, 1961).**

Serologic Methods for Detection of Antigen

The serologic techniques described above focus on antibody-detection; which has two inherent disadvantages (388): 1) there is always a delay between infection and development of a detectable level of antibody; and 2) antibody levels persist after clearance of an infection. Thus, false negative and false positive results often occur.

Direct immunologic detection of the antigen itself can be preferable. A serious drawback of this approach is that, compared to the presence of antibodies, antigen presence is a short-lived occur-

Figure 8-2.—Thin-Layer Immunoassay (TIA) Technique for Detecting Antigen or Antibody



Detection of antigen (I) or antibody (II) in a clinical sample:

- Either known antibody (I) or known antigen (II) is adsorbed onto a plastic plate.
- A clinical sample is added. If the sample contains the appropriate antigen (I) or antibody (II), an antigen-antibody complex is formed on the plastic plate.
- For the detection of antibody (II), a second antibody is added, which is an antibody to the antibody for which the test is done.
- A surface on which an antigen-antibody reaction has taken place is hydrophilic, i.e., it attracts water. The background is hydrophobic, i.e., it repels water. When exposed to steam, large water droplets form in the areas where the reaction has taken place, and small droplets form on the background.

SOURCE: World Health Organization, *Rapid Laboratory Techniques for the Diagnosis of Viral Infections*, Technical Report Series #661 (Geneva: WHO, 1981).

rence, especially for viral diseases. Current serologic methods for detecting antigen are described below.

1. Labeled immunodiagnostic reagent assays. The principal methods for detecting antigen are labeled reagent immunoassay that are modifications of the ELISA, RIA, or IFA tests for antibodies (described above) and employ a known specific, lab-prepared ***antibody*** as the first reagent to adhere to the plastic well. The ELISA technique for the detection of antigen is shown in figure 8-1.

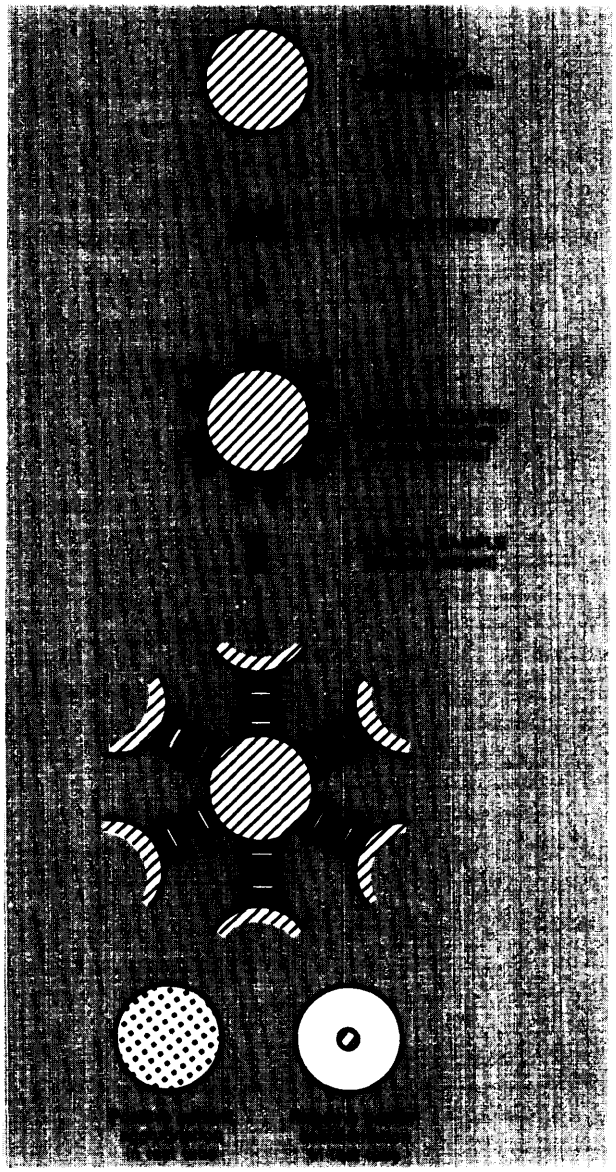
Labeled immunodiagnostic assays for detecting antigens have been applied to viral infections, amebiasis, toxoplasmosis, onchocerciasis (93), schistosomiasis (84), malaria, Chagas' disease, other protozoan infection (8), acute respiratory infectious (ARIs) caused by bacteria, and for detection of certain bacterial toxins causing diarrheal diseases. These tests can identify minute quantities of antigen, but their sensitivity has still been less than the sensitivity of direct examination techniques. As newer methods using MAbs are developed, sensitivity should improve greatly.

2. Agglutination assays. As indicated earlier, clumping of particles due to the interaction of antibody and antigen is the basis of all agglutination tests. In the following variations, the disease-producing organism or its byproduct (an antigen) is detected by complementary antibody fixed to particles which are added to a test sample.

In the coagglutination (COA) test, a bacterium ***(Staphylococcus aureus)*** is used as the earner particle for a specific antibody. When the antibody is mixed with a serum sample, it will agglutinate if the bacteria of interest are present. In a similar test, the latex agglutination (LA) test, latex particles are the earners. Technical considerations determine which method is superior for the diagnosis of any particular agent.

The reverse passive hemagglutination (RPHA) test, shown in figure 8-3, is used for rapid detection of viruses (smallpox, arboviruses, and hepatitis B). Here again, specific antibody is fixed to particles that agglutinate if the appropriate antigen is present in the test serum.

Figure 8-3.—Reverse Passive Hemagglutination (RPHA) Technique for Detecting Viruses



Detection of viruses in a clinical sample:
- **Known antibody is added to and adheres to the surface of treated erythrocytes (red blood cells).**
- **A clinical sample, suspected of containing the specific virus, is added. If the virus is present, complexes are formed linking antibody-coated erythrocytes together, visible in the test tube as an agglutination pattern.**

SOURCE: World Health Organization, *Rapid Laboratory Techniques for the Diagnosis of Viral Infections,* **Technical Report #661 (Geneva: WHO, 1981).**

Constraints on Serologic Diagnosis

Serologic diagnostic techniques provide some attractive technical options. Such methods can be very effective in epidemiologic surveys (203). Some of these methods have been adapted for field use in tropical areas (e.g., by absorbing a few drops of blood onto a filter paper strip for later examination in the laboratory). Serologic methods also are more easily automated than techniques of direct examination and thus have increased precision. Despite these features, however, very few such immunologic methods have proved to be of practical value in routine diagnostic laboratory practice. Most have remained in the research laboratory (388).

Several constraints limit the usefulness of serologic techniques in medical practice or for public health measures:

- Serologic procedures require sophisticated laboratory instruments and equipment. In many developing countries, basic services such as transport, electricity, and water are inadequate or unreliable, and trained personnel are lacking.
- In industrialized countries, where the expensive, sophisticated tests are feasible, few centers specializing in "exotic" diseases have the demand to justify doing the procedures. The result is that most medical facilities rely on the conventional techniques of direct examination.
- Parasite antigens are highly cross-reactive. Cross-reactivity, coupled with the presence commonly of more than one organism in an individual, makes the interpretation of serologic tests difficult.
- Test reagents vary widely, and different laboratories can report grossly divergent results using the same batch of antiserum. The need for standardization and for high-quality antigens is acute (177,388).
- "Paired sera" (from patients in the acute and convalescent stages of illness) may be required if changing antibody titer is a criterion for diagnosis.
- Many individuals are reluctant to have blood drawn.

Many of the antigens needed for serodiagnostic tests can be cultured in vitro. In vitro cultivation techniques are improving rapidly for many of the agents of tropical diseases (296). The other major source of precisely defined antigens for diagnosis will be recombinant DNA technology.

GENETIC TOOLS FOR IMPROVING DIAGNOSTIC TECHNIQUES

It is one thing for a university medical center to undertake the diagnosis of a single patient recently returned from the tropics and quite another for a survey team to apply diagnostic methods under field conditions to large numbers of local people in isolated, endemic, tropical areas. For use in the field, there is a great need for simple, reproducible, and inexpensive diagnostic methods that can provide a clear-cut indication of the status of an infection, and a specific identification of the causative agent. This need is especially acute because of the current work on developing vaccines for a variety of tropical diseases (see ch. 7). Without a means for accurate determination (or at least reliable estimation) of the prior exposure and immune status of vaccinees and controls, and their post-inoculation followup, valid field testing of these vaccines will not be possible.

Biotechnology (see ch. 5) is leading to the development of new diagnostic methods based on the use of MAbs and recombinant DNA (or recombinant RNA) techniques. These techniques, which are advancing rapidly, hold out a promise of accuracy and simplicity. In addition, they generally require smaller amounts of clinical samples than have heretofore been necessary. DNA and RNA hybridization tests hold great promise, but further development and testing are necessary before many of the tests can achieve widespread use in the field (137).

Monoclinal Antibodies (MAbs)

Immunodiagnosis using MAbs is building on the past 20 years of research in serologic diagnostics. The revolution is in making these diagnostic

test procedures much more sensitive and specific, more reproducible, faster, and more economical. Three diagnostic uses of MAbs are being developed (84):

- immunoassay of high specificity, i.e., making existing serologic diagnostic tests better by production of purer reagents;
- "two-site" immunoassay of antigen (the pathogenic organism itself or its byproducts) from appropriate body fluids, using two monoclonals directed against different binding sites of one antigen—one MAb to catch the antigen and another to label it for detection; and
- the use of cross-reactive MAbs to absorb certain common antigens out of crude mixtures, making possible the sensitive and specific detection of an antigen of interest.

MAbs have been used to distinguish between organisms that cross-react in conventional serologic tests, e.g., ***Trypanosoma cruzi*** and ***Leishmania braziliensis (71);*** between closely related species of New World ***Leishmania*** (283); between strains within single species of Theeileria (277) and ***Leishmania*** (145); between genetic variants (43) or life cycle stages (111) of ***Trypanosoma rhodesiense;*** and between larval and adult ***Schistosoma mansoni*** (332).

Assays of antigen in which MAbs are affixed to cellulose fibers or other solid substrates (e.g., plastic test wells) have been referred to as "dipstick technology" (136). Antigen present in blood, urine, stool, etc., binds to the MAbs, and the combination is detected by a second antibody through an ELISA-like color reaction. Immunodiagnosis using MAbs as probes for parasite antigen has been applied to such diverse conditions as tuberculosis (153), hydatid and other larval tapeworm diseases (78,79), Chagas' disease (9), onchocerciasis (93), and schistosomiasis (83,84,239). ELISA procedures using MAbs have been described for rotavirus infection (424), toxoplasmosis (8), and schistosomiasis (240). MAbs have been used to identify malarial organisms within mosquitoes without the need for exhaustive dissection and microscopy.

Nucleic Acid Hybridization Probes

The use of a nucleic acid (DNA or RNA) hybridization probe to identify the actual DNA or RNA of a disease-producing organism (112) is a promising diagnostic technique that has some important advantages, perhaps most important of which is high specificity. Scientists are now able to isolate and then reproduce ("clone") DNA segments from known organisms, label the segments radioactively, separate them from the normal double-stranded state to a single-strand state, and then test them against unknown specimens of DNA (e.g., in a stool sample) for the ability to hybridize (re-form double-stranded DNA). If the suspected organism is present, then the labeled DNA and the sample DNA, being the same, will hybridize. The radioactivity or fluorescence or enzymatic color change can then be detected.

The nucleic acid hybridization techniques for different organisms vary, but such techniques can be developed to be simple, practical, and relatively inexpensive. Using these techniques, investigators can screen large numbers of samples at one time. Samples can be collected and stored for several weeks before the test is completed. Nucleic acid probes should be useful for large-scale epidemiologic and surveillance studies (117,246). The major disadvantages of the tests that use radioactive labels are the radiation hazard and the short shelf-life of the radiolabeled reagents. These problems can probably be circumvented in most cases by substituting tests that use color change methods in place of radiolabels (196,202).

The uses of nucleic acid hybridization techniques to detect malaria parasites (122), ***Leishmania*** spp. (409), ***Escherichia coli*** (246), and rotavirus, and to detect Salmonella bacteria in food products (116) are described below. The utility of nucleic acid hybridization probes in the field in developing countries must be further evaluated, but good results have been reported in detection of rotavirus and ***E. coli*** in stool sample dots. Although nucleic acid hybridization is still in its early development phase, it may well progress to a predominant role in diagnosis.

DIAGNOSIS: CURRENT STATUS FOR SELECTED TROPICAL DISEASES

Malaria

Since the emergence of drug-resistant malaria parasites in the late 1950s, the importance of specific diagnosis, including a determination of whether the malaria parasites are drug resistant, has been greatly elevated. Previously cheap, safe, effective, and completely standardized antimalarial treatment regimens were available, and, presumptive treatment, without definitive diagnosis, was freely administered. Now, however, appropriate treatment rests on accurate diagnosis.

Conventional Diagnosis

Diagnosis of malaria based on physical examination alone can be difficult, because malaria's symptoms are protean. For that reason, laboratory diagnosis of malaria is important. The standard method of diagnosis is by microscope examination of a stained blood smear made from a finger-prick. The presence of malaria parasites is definitive. Under field conditions, there are generally quite a few false negatives, because people with malaria do not always have large numbers of malaria parasites circulating in their blood. In the absence of microscopically confirmed infection, a presumptive diagnosis of malaria may still be made, based on clinical symptoms. Treatment is given to reduce the parasite load of the population and, in effect, to prevent mosquitoes from acquiring malaria.

Although methods for the serologic diagnosis of malaria have long been available (CF, IHA, and IFA tests), they are not widely used for two reasons. First, the need for equipment and materials to perform the tests cannot always be met. Second, the tests demonstrate the presence of antibodies, which persist after cure and may indicate previous infection. In an endemic country where infection is always possible, such tests do not greatly help the decision to treat. Nevertheless, these tests have been useful for epidemiologic surveys (especially IHA and IFA tests (168)), and for special purposes such as establishing the presence of antibody in a particular patient in whom infection is suspected but cannot be demonstrated directly (e.g., in a blood donor who is suspected of having transmitted malaria to a blood recipient).

Recent Progress

Serologic Diagnosis.—Improved serologic methods for the detection of blood stage malarial antigens are being developed: RIA and ELISA tests for the detection of parasitized red blood cells have been developed. Sensitivity of parasite detection with these tests is encouraging, and reproducibility should improve as standard reagents become available (353).

In the hope of establishing standard reagents for malaria serology, the World Health Organization's (WHO) Immunology Research and Training Center, Geneva, has established a registry of malarial MAbs collected from other laboratories and evaluated for potential value as serodiagnostic reagents.

Sporozoite Diagnosis.—Immunologic work on plasmodial sporozoites has led to the development of two methods to test for sporozoites in mosquitoes. Such testing has importance for epidemiologic studies in determining the degree to which various mosquitoes function as vectors of species of malaria parasites.

In one method, MAbs are used to identify sporozoites in mosquito squashes by a direct binding assay, which is species-specific. This method was developed as an RIA, but an ELISA procedure is being developed as well. Although the test procedure must be carried out in a centralized laboratory, the samples are stable without refrigeration and easily handled, and the results are available within an acceptable time interval for epidemiologic purposes.

A second method, another new immunoradiometric test (inhibition of idiotype-anti-idiotype interaction, or "4 i-assay"), has been developed to detect circumsporozoite protein (280) and is based on inhibition of binding of two MAbs. The first MAb is against malarial antigen, and the second MAb is against the first MAb (thus called an idiotype, which resembles the original antigen). When both MAbs are mixed in a test well, they bind to-

gether in a detectable way, unless the test sample contains antigen. If the sample contains antigen, the antigen will bind specific antibody, thus inhibiting the two MAbs from interacting (inhibition indicates the presence of true parasite antigen). This test is sensitive enough to distinguish between different species of malaria parasites. The method has general applicability. Furthermore, because the two immunoglobulin reagents are MAbs, this method does not require cultivation and purification of antigen from parasite, and the two immunoglobulin reagents are completely pure (422).

DNA Hybridization.—Work on development of a rapid diagnostic test using specific DNA hybridization is proceeding. A recent publication describes experimental success in identifying ***Plasmodium fa]ciparum*** parasites in samples of blood from in vitro culture and from malaria patients (122). This method is still at a very preliminary stage in relation to any practical use, because the sensitivity is no better than microscopic examination of a stained blood film, and the procedure involves a number of laboratory steps that take about 24 hours to complete.

In Vitro Cultivation of Malaria Parasites and Microtest of Drug Sensitivity .-With the current situation of widespread resistance to drugs by malaria parasites, diagnosis of drug susceptibility in parasite isolates is an important epidemiologic task. The method for in vitro cultivation of ***P. falciparum,*** the malaria species with widespread drug resistance, has been adapted to several techniques for testing drug susceptibility against the primary antimalarial. The tests are available in kit form from WHO.

Research Needs

Greater standardization of serologic tests for the detection of malaria antigens and antibodies is needed. These tests need to be adapted for field applications, both as quick and easy diagnostics at remote or poorly equipped treatment posts and to assess any vaccination trials that may be attempted in the future. Assessment of vaccination trials will rely on detecting antibodies in individuals who did not have antibodies before vaccination as well as determining infection rates post-vaccination.

A quick and easy field method is needed to establish the existence of infection (to conserve drug for true cases) and to differentiate species of ***Plasmodium*** (for appropriate drug type). Similarly, a field method to detect infection in mosquito vectors is needed to assess the impact of vaccination on the overall risk of transmission.

A less urgent but important need is a method of screening blood bank donations to prevent transfusion malaria.

Schistosomiasis

The major schistosomes infecting humans are ***Schistosoma mansoni, S. japonicum,*** and S. ***haematobium.*** Current control for schistosomiasis calls for the identification and treatment of all infected persons at regular intervals (usually 6 months) (353). Quick and reliable diagnosis is essential to identify people infected and for assessing the effectiveness of the treatment. To determine optimal treatment, it is necessary not only to diagnose the presence of infection but also to determine how heavy the parasite load is.

Conventional Diagnosis

Direct examination of feces or urine for characteristic eggs is the classical method of diagnosis of schistosomiasis. There are a number of methods for concentrating the sample to maximize the chance of detection and a number of methods for accurately estimating the number of eggs in order to estimate the parasite burden in the host. Cytoscopy or sigmoidoscopy (viewing through instruments inserted into the body) is occasionally used to detect lesions, and rectal biopsy is sometimes used. There is a CF test, an intradermal skin test, and the COPT to help confirm diagnosis.

Recent Progress

Two new techniques have been developed for S. ***haematobium*** diagnosis. One is the filtration of urine using a reusable plastic woven filter which isolates excreted eggs. The second is a prototype image processing and pattern recognition apparatus for automated S. ***haematobium egg*** counts that has been tested in the laboratory and is undergoing field trials in endemic areas (353).

A new diagnostic kit has recently been made available by the Program for Appropriate Technology in Health, a nonprofit, nongovernmental organization. The kit is for the diagnosis of S. ***haematobium,*** and it is designed for quick, practical, and reliable field use.

In a special collaborative study sponsored jointly by WHO and the Edna McConnell Clark Foundation, eight research laboratories evaluated a number of procedures for immunodiagnosis of schistosomiasis (247). Included were the COPT, ELISA, IHA, IFA, RIA, and other procedures, testing a pool of banked sera against a variety of schistosomal antigens. A study of this type is of great value in developing and standardizing materials and methods for immunodiagnosis.

Several candidate antigens for immunodiagnosis have been identified (59). There are several modifications of the ELISA procedure, including an inhibition-ELISA used to detect and characterize schistosomal antigens (l). Many laboratories have developed MAbs for identification of relevant antigens or for species diagnosis (e.g., 1,83,84,99,237,238,239,240) .

Many workers are attempting to identify and isolate relevant protective antigens, and at least half of those are actively engaged in gene cloning experiments. Several laboratories are devising improved diagnostic methods, primarily with ELISA-based tests. Most schistosomiasis experts believe that effective MAb-based diagnostic tools are very near or within 5 years of introduction (16).

Research Needs

Field methods for quick and easy diagnosis of schistosomiasis are being introduced but still require evaluation and standardization.

Trypanosomiasis

African Sleeping Sickness (African Trypanosomiasis)

There are two forms of African sleeping sickness in humans. ***Trypanosoma brucei gambiense*** causes the chronic form found in west Africa. T. ***b. rhodesiense*** causes the acute form in east Africa and also infects livestock over large areas of the continent.

Conventional Diagnosis.—Direct examination for African trypanosomes is done from stained blood preparations, but parasites are extremely difficult to find. In the chronic form (T. ***b. gambiense),*** fluid drawn by needle from lymph nodes in the neck is examined. The number of parasites detected varies daily. In the acute form (T. ***b. rhodesiense)*** too, parasites may vary in density, making diagnosis difficult. Cerebrospinal fluid may also be examined. Inoculation of laboratory animals or culture on appropriate media to allow the parasites to multiply is sometimes useful.

Immunodiagnostic techniques have been available for many years (IFA and CF tests), but these must be performed in central laboratories. The ELISA has also been found effective in the laboratory but not for the field (353). All of these tests are valuable for epidemiologic studies, but the delay in reaching a diagnosis limits their usefulness for patient care.

Recent Progress. —Tests for antibodies against T. ***b. gambiense,*** which can be read within minutes and carried out with blood obtained from a finger-prick, are under development and evaluation in the field. They are the card agglutination test for trypanosomiasis (CATT), the Cellognost test (a commercial technique based on indirect haemaggIutination), and the Tryptest. CATT is being evaluated on a large scale in west Africa. This test has been found to be as specific as the IFA test. It is easily transported to the field, requires little technical skill, and gives results within minutes. The lack of stability of the antigen and storage under field conditions are problems to be worked on. Antigens to diagnose T. ***b. rhodesiense*** are being sought (353).

Two other techniques are also being evaluated: the miniature anion exchange column technique and the microhaematocrit buffy coat centrifugation method. Both have shown greater sensitivity than blood film examination (208). Development of the double centrifugation technique for the detection of trypanosomes in cerebrospinal fluid has improved diagnosis of central nervous system involvement (353).

The three subspecies of the ***Trypanosoma brucei*** species complex are morphologically indistinguishable. Two species (T. ***b. rhodesiense*** and T. ***b. gambiense)*** are infective to humans, causing sleeping sickness; the third (T. ***b. brucei)*** is infective to wild and domestic animals but not to humans. Scientists' inability to distinguish between human and animal forms has epidemiologic importance for determining risk to humans where animals are found infected. Culturing of trypanosomes in human serum is widely used for determination of infectivity to humans. Isoenzyme electrophoresis, DNA hybridization, and comparative IFA with standardized sera have also been used recently.

Research Needs.—Several avenues of research may be nearing fruition for the diagnosis of African trypanosomiasis. There is a need to develop sensitive and specific techniques suitable for field use.

Chagas' Disease (American Trypanosomiasis)

Chagas' disease, caused by the protozoan parasite ***Trypanosoma cruzi,*** affects more than 12 million people in Latin America (229). Acute and chronic phases of the disease vary somewhat from one region to another (166). Most damage is done by tiny, nonflagellated forms within the cells of heart muscle and certain nerve ganglia.

Conventional Diagnosis.—The clinical picture of Chagas' disease is variable. Examination of blood during the acute phase of the disease may reveal parasites. Because of commonly low parasite density in blood, several culture techniques are used to allow the parasite to multiply to a detectable level: inoculation into laboratory animals followed by periodic examination over 60 days; in vitro culture of blood; xenodiagnosis (allowing clean, uninfected reduviid bugs to feed on the suspected patient and then examining the hindgut of the bug for trypanosomes after 2 weeks). All these methods often require repeated attempts.

A CF test (the Machado-Guerreiro test) using T. cruzi antigen is available, as is other serologic diagnosis for the direct detection of antibody. Conventional serologic tests for Chagas' disease cross-react with leishmaniasis, leprosy, and syphilis antigens and are not sufficiently sensitive to detect Chagas' disease with assurance. A number of groups are working on development of an immunodiagnostic reagent, and optimism is generally high. Immunodiagnosis of specific antibody is available as a procedure in centralized laboratories, but standardization between laboratories is a problem.

Recent Progress.—Standardization of serodiagnostic techniques has been promoted by the Special Program for Research and Training in Tropical Diseases (TDR). A serum reference bank is now providing standardized lyophilized (freeze-dried) serum samples to laboratories throughout Latin America, and a network of collaborative laboratories has developed protocols for the standardization of reagents, techniques, and procedures (353).

Many workers have developed MAbs that may be useful for the detection of various trypanosome antigens (9,259). Development of ELISA diagnosis is under way in a number of laboratories. Investigators have shown that circulating antigens in acute infections can be detected (7).

A specific diagnostic test is being evaluated using purified cell membrane antigens of T. ***cruzi*** which are fixed on polyamide strips. After exposure to suspected serum, the strips are treated as in ELISA-type tests to detect any antigen-antibody complexes that would form if the serum were from a person with Chagas' disease. Preliminary results indicate that the test greatly reduces, but does not eliminate, nonspecific reactions.

Blood-transfusion-transmitted Chagas' disease in Brazilian hospitals is a serious problem, and better screening methods for donated blood are needed. An agglutination test for rapid screening of donated blood for T. ***cruzi*** infection is undergoing evaluation in Brazil, where it was developed, and in a network of collaborating laboratories (353).

A DNA-DNA hybridization probe shows promise of detecting the presence of T. ***cruzi*** organisms or DNA fragments present in the blood. T. ***cruzi,*** has a unique, highly variable type of DNA (kinetoplast DNA) that can be used to distinguish species and strains within species. A technique for isolating DNA, cutting it into pieces, and then sep-

arating the DNA into recognizable patterns (restriction endonuclease finger-printing of kinetoplast DNA) promises to provide a valuable new tool for epidemiologic and clinical purposes. New subdivisions of T. ***cruzi*** strains can be demonstrated using this method. These refinements in the taxonomy of the parasites may help to explain the variability of the disease in different localities, including the variation in clinical symptoms and the variable susceptibility or resistance in hosts (353).

Research Needs.—A quick, easy, and reliable method is needed for diagnosis of acute Chagas' disease (when treatment might be prophylactic, and because the treatment is toxic). A means to predict prognosis in different geographic areas also is desirable.

A sensitive test for screening donated blood is also particularly important in Chagas' disease, as infection by transfusion is a serious problem in endemic areas.

Leishmaniasis

Leishmaniasis is a disease with three clinical presentations depending on the leishmanial parasite species. Cutaneous leishmaniasis, caused by either ***L. tropica, L. mexicana,*** or ***L. brazilienis*** (depending on geographical location), is a self-limiting and usually self-resolving sore at the point of infection. Mucocutaneous leishmaniasis, caused by ***L. brazilienis,*** begins as a sore but commonly metastasizes and proliferates in the nasal and pharyngeal mucous membranes. Visceral leishmaniasis, or "kala-azar," is caused by ***L. donovani*** and affects the spleen, liver, bone marrow, and lymph glands.

Conventional Diagnosis

Diagnosis of leishmanial organisms is complicated by the rather uniform appearance of different species under the light microscope, and cross-reactivity of different species with conventional serologic diagnosis. Until recently, microscopic identification and conventional serologic techniques were the only techniques available for diagnosing leishmaniasis.

Photo credit: Office of Technology Assessment

Leishmanial organisms as seen through a light microscope.

The inability to distinguish correctly between species of ***Leishmania*** can have serious consequences for patients. For example, the lesions of ***L. mexicana*** and ***L. braziliensis*** are very similar at first appearance, and both species overlap in many parts of South America. Without treatment, ***L. mexicana*** is self-resolving, but ***L. braziliensis*** progresses to gross destruction of the nose and throat. Thus, treatment and followup for ***L. braziliensis*** infection are critical. But the treatment is itself highly toxic and clearly not to be used for those not requiring it.

Recent Progress

Investigators have developed a DNA hybridization probe to distinguish between ***L. mexicana***

and ***L. brazdiensis*** (409) using kinetoplast DNA, a unique form of DNA in ***Leishmania*** and ***Trypanosoma*** species. Kinetoplast DNA is extracted from growing cultures of various species of ***Leishmania*** organisms, processed and labeled radioactively. Test material is collected from the patient as "touch preparations" on nitrocellulose filter paper from suspected lesions. Hybridization and analysis are then carried out. Kinetoplast DNA hybridization is highly species-specific and provides a relatively rapid means of diagnosis direct from infected tissue. This has now been tested for the diagnosis of human patients and shows promise.

Another test, called the "DOT-ELISA" test, has been developed, and represents a major advance in the rapid field diagnosis of visceral leishmaniasis. It also is useful for field surveys for identifying infected vectors (267).

In recent years, scientists have prepared MAbs against a variety of antigenic determinants in ***Leishmania*** species (71,91,139,145,171) and used them to probe for specific morphologic and taxonomic differences. Among the many monoclonals produced, some recognize antigens common to all kinetoplastid ***(Leishmania*** and ***Trypanosoma)*** species tested; others bind only to certain strains within a single species. The process of sorting out these specificities and defining the precise nature of the reactive leishmanial antigens should produce advances in knowledge and diagnosis of these organisms.

Research Needs

The diagnosis of ***Leishmania*** species is very important, because different species produce similar lesions but have very different long-term consequences. DNA hybridization looks promising but needs development for practical use.

In the clinical-epidemiologic area, there are many strains and types of ***Leishmania,*** but a lack of a good classification system. More field surveys to determine prevalence of disease are needed, and those surveys will require practical field tests.

Filariasis

Filariasis is a collective term for several distinct parasitic infections by insect-transmitted, tissue-dwelling nematodes. The principal species are ***Wuchereria bancrofti*** and ***Brugia malayi,*** which cause filarial elephantiasis; and ***Onchocerca volvulus,*** the cause of onchercerciasis (river blindness) in west Africa, also found in Central and South America.

Conventional Diagnosis

Conventional diagnosis of filarial infections has severe limitations. For lymphatic filariasis ***(Wuchereria*** and ***Brugia),*** diagnosis is made by microscope examination of stained blood films to find microfilariae. Density of microfilariae is usually very low, especially during the early stages of infection; and for several species, it is cyclical according to a circadian rhythm, so at times there may be no microfilariae present in the blood. Diagnosis of onchocerciasis (in which the subcutaneous tissues are infected) is made by using a special surgical punch to take tiny snips of skin and then examining this skin under the microscope. Because of cross-reactions with other organisms, no dependable serologic diagnostics for filarial infections are available.

Photo credit: H. W. Brown and F. A. Neva, "Basic Clinical Parasitology," Appleton-Century-Crofts. Reprinted by permission.

Microfilariae of *Onchocerca volvulus* were **found** in skin snips from these nodules.

Recent Progress

A number of MAbs to filarial antigens have been produced, but immunodiagnosis has been hindered by a high level of cross-reactivity with other helminth antigens. Excretory-secretory antigens, which are released by the parasite and circulate in the host's blood, and surface antigens on the parasite itself are being assessed for use in immunodiagnosis of filaria. The demonstration of circulating antigens in ***Onchocerca*** infections has made a diagnostic MAb feasible. A good correlation between circulating antigen and presence of parasites was found in one study, using a MAb in an RIA (93). However, the false positive rate was high, suggesting that further refinement of this test is needed. At a workshop held in 1983, 26 researchers from around the world brought about 50 antifilarial MAbs representing the total successful effort to that date (16).

Tests for detection of specific antibodies against filarial worms, for circulating parasite antigen, and for parasite surface antigen are being developed. There is optimism about the possibility of a breakthrough in filarial detection within the next few years. At least one Federal Government laboratory, one in industry, and one in a university are conducting or planning recombinant DNA work for expression of such filarial antigens.

A problem in diagnosing filariasis is the identification of parasites found in wild-caught vector insects. The problem of confusing intermediate life stages of disease-producing organisms with other organisms of little public health significance is common to vector-borne diseases, and represents a special problem in diagnosis which is amenable to solution by modern methods. In areas of west Africa with active vector control programs against onchocerciasis, new insects with potential vectorial capacity are entering controlled areas from adjoining regions. Some of these insects are naturally infected with filarial larvae, possibly parasites of wildlife, that cannot be distinguished by direct examination from larval ***Onchocerca.*** The development of probes, either by MAbs or possibly DNA hybridization, would permit identification of these nematode larvae and determination of whether they pose a threat to humans.

Research Needs

Very generally, improvements in all aspects of the diagnosis of filarial infections are needed. Because much remains to be learned about the epidemiology and pathology of filariasis, useful diagnostic tests are needed. It is important both for patient care and for research purposes to be able to distinguish animal and human filariae, a task which at present is not always possible.

Leprosy (Hansen's Disease)

Leprosy, caused by the bacillus Mycobactetiuzn ***leprae,*** is the only bacterial infection among the six diseases targeted by TDR. Since the 1950s, the WHO-recommended strategy of leprosy control through early case finding, followup of contacts, and chemotherapy of patients has proved to be difficult to implement and sustain in many countries (353).

Conventional Diagnosis

The initial diagnosis of leprosy is through recognition of areas of skin that lack feeling (anesthesia) and may be discolored or slightly raised. Definitive diagnosis is made by acid-fast staining and microscope examination of skin biopsy smears from these suspected lesions. This technique is useful in all forms of leprosy, but there maybe few bacteria in milder cases, making detection uncertain.

The lepromin or Mitsuda test, a skin test similar to the tuberculin skin test (see below), is used as a prognostic test after leprosy is diagnosed. It tells where the patient is along the immunopathologic scale of disease. A crude suspension of killed bacilli (derived from human leprosy patients) is injected under the skin of the patient. About 3 weeks later, the skin reaction is assessed, allowing a prognosis to guide treatment.

A lymphocyte transformation test has been available for a decade as an indicator of ***M. leprae*** infection and the potential course of the disease (133). This test is an indicator of cell-mediated immune response. Despite its technical difficulties and subjective interpretation, the lymphocyte transformation test has given useful results in experimental field studies of exposure to leprosy an-

tigen, though it has not become a practical method for routine use. Another test of the cell-mediated immune response, microphage migration inhibition, is also not used routinely (168). It also possible to isolate and diagnose leprosy by inoculation of a clinical specimen from a suspected case into the footpad of a mouse.

Recent Progress

A phenolic glycolipid molecule has recently been isolated and identified as a unique and specific antigen that is abundant on ***M. leprae*** and in the skin lesions of leprosy patients. This antigen has been used in an ELISA and has proved to be specific for antileprosy antibody (56). It has been tested experimentally to detect antibody in the blood of leprosy patients, with excellent results (433). This antigen has the potential to be used in a specific diagnostic test for leprosy. Investigators have found that contacts of known cases are more likely than other people to develop antibodies to this antigen. The phenolic glycolipid test may therefore be useful in screening persons at increased risk of developing clinical disease.

A number of apparently ***M.*** leprae-specific antigens (other than the phenolic glycolipid antigen) have been reported (30,41,149), and MAbs have been produced, which may also prove useful as diagnostics. Serologic tests using IFA, ELISA, or RIA are being developed for epidemiologic studies. The fluorescent leprosy antibody absorption test has shown high specificity and is being evaluated for predictive value in long-term epidemiologic studies (2). Skin tests have been developed for monitoring delayed-type hypersensitivity reactions to ***M. leprae*** after immunization, though the current test antigens are crude extracts that lack specificity. Other skin test preparations using soluble antigens are being evaluated in the field for predictive value (224,232,346).

Research Needs

A method of culturing ***M. leprae*** in vitro is an urgent need. Such a method could lead to improved methods for early diagnosis, which in turn would lead to earlier treatment and favorable prognosis. Practical techniques to diagnose leprosy for epidemiologic studies need to be developed to allow better understanding of transmission and susceptibility.

Progress in development of serologic tests that are sensitive and specific has raised optimism about prospects for achieving practical diagnostic techniques for leprosy. The phenolic glycolipid antigen is most promising at present and may be developed into a useful diagnostic test. At present, the main source of this antigen and others is from ***M. leprae*** grown in armadillos. Obtaining antigens from ***M. leprae*** grown in armadillos is a slow process. It may be necessary or useful for diagnosis to make important molecules from ***M. leprae*** using recombinant DNA.

A method for the differentiation of patients with lepromatous leprosy (the severe form with poor prognosis) and tuberculoid leprosy (the mild form with good prognosis) is needed to improve understanding of the epidemiology of the disease and for the early recognition of individuals at high risk of developing disease. The evidence for a genetic basis to resistance needs further study.

Tuberculosis

Tuberculosis remains a major threat to health in many parts of the world, causing several million deaths annually. Tuberculosis control programs are built around vaccination coupled with early case detection, treatment, and followup of active cases. Diagnostic methods and early diagnosis before symptoms are overt are critical to the control of this disease.

Conventional Diagnosis

The consideration of clinical symptoms and examination of direct sputum smears for the presence of the causative tubercle bacilli are the conventional means of diagnosing tuberculosis. If no bacilli are found in a direct smear but tuberculosis still is suspected, culture isolation of bacteria from a clinical specimen (usually sputum, though urine, spinal fluid, or tissue biopsy may be appropriate) is attempted. Culturing also is used to confirm a diagnosis made by a direct smear. Isolation of bacilli from culture is the preferred method, but several serious problems arise: the need to decontaminate the specimen to prevent

overgrowth by contaminating bacteria from the oral cavity; the need to collect and culture multiple samples from a suspected case; and the slow multiplication of the bacilli, which means that it may take 3 to 6 weeks for growth to appear.

Chest X-rays continue to be of great value in the diagnosis of tuberculosis, particularly in areas where other diseases (like histoplasmosis) with similar X-ray appearance are absent. X-rays are particularly useful for determining (by noting differences between earlier and later films) that a dormant case has been reactivated.

Microscope examination of stained sputum smears can be used to quickly identify tuberculosis-like bacteria, but does not distinguish virulent tubercle bacilli from look-alikes. In areas of the world where active pulmonary tuberculosis is common, a presumptive diagnosis can be made on the basis of numerous bacteria with particular staining qualities (acid-fast) in the sputum. It is clear, however, that many different species of ***Mycobacterium,*** in addition to ***M. tuberculosis,*** can infect humans and cause tuberculosis-like disease. Such environmental mycobacteria (called "atypical mycobacteria") are diagnosed by their characteristics in culture, and cannot be distinguished on direct sputum examination. Some of these (the ***M. avium-intracellulare*** complex) are associated with acquired immunodeficiency syndrome (commonly known as "AIDS").

The tuberculin skin test is used to identify people infected with tubercle bacilli, by means of an allergic reaction to tuberculosis antigens (delayed-type hypersensitivity). A small amount of tuberculoprotein is introduced into the skin, and the person is observed for an inflammatory reaction 2 to 3 days later. Old tuberculin (a crude concentrate of tubercle bacilli in culture medium) has been replaced by purified protein derivative (PPD), a purer, more standard material.

Three techniques can be used to introduce the tuberculin: 1) the Mantoux test, in which PPD is injected intradermally; 2) the Vollmer patch test, in which tuberculin is applied to the skin on a gauze adhesive strip; and 3) the Tine test, in which the tuberculin is dried ***on*** the points of a standard puncture device that is pressed into the skin.

All of the tests have advantages and disadvantages. None of these tests can be used to test anyone already vaccinated against tuberculosis (a large percentage of the population in many developing countries and in a number of developed countries), because vaccinated individuals should react to the challenge. The Mantoux is the most sensitive and reliable test, because a standard volume and amount of tuberculin is injected directly into the skin; however, the procedure of injection by hypodermic is a practical disadvantage. The Tine test is a rapid and easy test for use in large population groups, but it gives a relatively high number of "false positives" (people who test positive but who actually are not infected); positives must be followed up by a Mantoux test. The Vollmer patch testis useful for skin-testing infants and children, but it is less sensitive than the Mantoux test.

A positive tuberculin skin test may be caused by infection with other species of mycobacteria or as a result of previous vaccination. A negative tuberculin test is strong evidence against the tuberculosis diagnosis, but can also result from loss of potency in stored PPD, and is occasionally observed in far advanced cases.

Recent Progress

ELISAS have recently been applied to the diagnosis of active tuberculosis pulmonary infection (183). Application of recombinant DNA methods to ***M. tuberculosis*** is in its infancy, but several investigators are planning projects. In a handful of laboratories including the National Institutes of Health and several academic institutions, MAbs are being reacted with ***M. tuberculosis*** to isolate and purify antigens that may be useful as diagnostic targets and may be the basis for a new tuberculosis vaccine.

Attempts are being made to isolate specific antigens for skin tests in an attempt to make tuberculin skin tests less cross-reactive to infections with other types of mycobacteria. Several tuberculosis researchers also work with ***M. leprae,*** the related bacterium that causes leprosy.

Research Needs

Tuberculosis may be the most serious infectious disease problem in many developing countries, as much a social as a scientific dilemma. There is an obvious need for better vaccination and diagnosis. Diagnostic methods are needed to differentiate the "atypical mycobacteria, " such as ***M. intracellulare*** and ***M. smegmatis,*** which hinder diagnosis and seem to interfere with the effectiveness of BCG (Bacillus Calmette-Guerin) immunizations.

Diarrheal and Enteric Diseases

Diarrhea] and enteric diseases are caused by a variety of viruses, bacteria, protozoa, and worms, and a single individual often is infected with several at one time. Full understanding of the various etiologic agents will come only when simpler techniques of diagnosis are available. Clinical diagnosis of diarrheal disease calls for immediate institution of therapy. Fortunately, dehydration therapy is appropriate for all diarrheal disease (see ch. 9). Nonetheless, identification of specific pathogens is essential for understanding the distribution of different agents and to establish priorities for specific actions.

Viral Infections

Viruses are now recognized as important agents of gastroenteritis. The principal agents are rotaviruses and Norwalk agents, though adenoviruses, astroviruses are enteroviruses, coronaviruses, and calciviruses are also found in fecal specimens (15).

Conventional Diagnosis.—Most of the viruses that cause diarrheal and enteric diseases do not grow under ordinary cell culture conditions, so direct diagnosis of viral antigen in stool specimens or detection of a serologic response is necessary. Electron microscopy is a sensitive and simple method for detecting the presence of virus (if an electron microscope is available), but only limited numbers of samples can be processed.

A variety of immunologic methods have been developed for detection of viral antigen in stool samples. The ELISA, IFA test, RIA, and CIE produce good results, though ELISA and RIA have the greatest sensitivity.

Recent Progress.—Progress has been achieved in isolating and cloning rotavirus DNA and then developing DNA hybridization assays (117). Cloned DNA hybridization probes have been used on stool specimens for rotavirus diagnosis. The utility of DNA hybridization probes in the field in developing countries must be further evaluated, but good results have been reported in detection of rotavirus in stool samples taken in remote areas of Venezuela (146).

A test "kit" for rotavirus based on an ELISA has been developed and evaluated and is now in use by more than 50 investigators in the field. A second generation ELISA test based on the use of MAbs is being developed (427).

Bacterial Infections

Conventional Diagnosis.—Definitive identification of bacteria causing diarrhea is by the isolation of the agent through culture of clinical samples (e.g., stool samples for ***Shigella*** and ***Vibrio cholerae;*** blood or stool samples for ***Salmonella.*** Culture of ***V. cholerae*** is relatively simple and gives a result in 18 hours.

Serologic diagnosis of ***Salmonella typhi,*** the cause of typhoid fever, is possible because specific agglutinins appear in the blood at 7 to 10 days of illness (the Widal reaction). For cholera, rise in titer of specific agglutinins or antibodies confirms the diagnosis.

Two tests for the isolation and identification of enterotoxigenic E. ***coli*** have been available for a number of years: one uses a miniculture of adrenal cells (302), and the other uses suckling mice (90).

Recent Progress. —Although E. ***coli*** is one of the most intensely studied of all organisms, with many thousands of research publications on all aspects of its biology and biochemistry, much remains to be learned about its relation to diarrheal disease. The several strains of E. ***coli*** that produce diarrheal and intestinal disease in humans are recognized and described on the basis of clinical pathology ("enterotoxigenic," "enteropathogenic," "enteroinvasive," see ch. 4). All of these characteristics are under genetic control, and investigators in several laboratories are identifying and cloning the genes that code for attachment and

colonization, virulence, toxin production, and antibiotic resistance. With the genes in hand, diagnostic procedures can be developed.

DNA hybridization probes for field identification and typing of E. coli are under development in several laboratories. A DNA hybridization test for identification of enterotoxigenic E. coli has been available for several years (41,42) and now has been tested in the field (103,312). The DNA hybridization test has been shown to be very specific, reliable, stable, and sensitive (1,000 times more sensitive than the standard assays). It appears to be a valuable tool for epidemiologic studies.

The WHO Control of Diarrheal Diseases (CDD) program has evaluated the "Biken" gel diffusion test for detection of certain strains of ***E. coli.*** The test is simple to perform, accurate, reproducible, and has potential for use in developing countries. Commercial production and marketing of the materials and reagents used in the test is being pursued (427).

The CDD program is also evaluating ELISAS for diagnosis of strains of ***E. coli.*** Results indicate a potential for widespread application, though further development is needed to make it suitable for routine diagnostic use (427).

A DNA-DNA hybridization probe was used by one team of researchers to detect ***Salmonella*** bacteria not in stool samples but in food products (116). This type of application may provide far more serotype-specific identification of contaminating organisms than conventional culture methods, without the need for incubators, sterile media, and glassware.

Cholera is being studied by four or five researchers in the United States. Recombinant DNA libraries are being constructed to collect gene sequences from wild-type organisms. Studies are under way on the transmissible genetic elements isolated from ***V. cholerae*** from endemic areas such as Bangladesh, and differences between toxigenic and nontoxigenic organisms are being defined (16).

Campylobacter jejuni is now recognized as an important diarrheal agent, but epidemiologic study is hampered by lack of a serotyping technique. Antigenic studies are under way to develop a serotyping system. A simple slide agglutination technique to identify antigens is under evaluation in a number of developing countries (49).

Acute Respiratory Infections (ARIs)

ARIs are caused by a range of etiologic agents—viruses, bacteria, rickettsia, and parasites—presenting great diagnostic complexity. Although most of these agents can be specifically diagnosed, the process normally requires multiple serologic tests of various kinds.

Many diagnostic procedures based on isolation and culture of the organism or based on comparison of an initial and a later serum sample ("paired sera") do not provide timely enough information to be relevant to individual patient care. Also, to a large degree, the diagnosis of ARIs can be and is made on the basis of clinical symptoms, because no matter what the diagnosis, specific therapeutics are lacking, especially for the many viral diseases.

Because much of the treatment of ARIs consists of providing symptomatic relief, the identification of specific disease-causing agents is not so important. In lieu of specific diagnosis flowcharts or decision trees, using a patient's symptoms as criteria may be more appropriate for use by medical staff at all levels of the health systems of developing countries.

Still, in many cases, the etiologic agent needs to be identified. For the treatment of the individual patient, diagnosis permits rational use of available therapy. The nonspecific prophylactic or placebo use of antibiotics for any undiagnosed ARI is greatly abused. Bacterial infections can be treated with common antibiotics, but for viral agents, only symptomatic support and relief are proper (with the exception of viral influenza for which an antiviral drug is available).

For public health needs, specific diagnosis is necessary to assess, plan, implement, and evaluate interventions against epidemic outbreaks and endemic infections.

Conventional Diagnosis

Faced with an individual patient with respiratory infection, the clinician or epidemiologist must

make simplifying judgments to decide which diagnostic procedures are in order. Simple microscope examination of nose or throat secretions can be useful, though limited, in ruling out possible etiologic agents.

Culture and isolation of the pathogen leads to a definitive diagnosis, but the growth takes time (usually at least 2 days), delaying the diagnosis. For viral agents and even bacterial agents, this procedure requires careful collection; special handling; storage and transport; tissue culture facilities (including appropriate culture media, and cell types for virus growth); and equipment for serologic diagnosis (e.g., immunofluorescent microscope, scintillation counter, electrophoretic equipment). Prior antibiotic treatment or contamination of the sample at any of several points from collection to inoculation, in the mature medium can easily lead to incorrect results.

Serologic diagnosis by most of the standard tests can demonstrate specific antibody in the individual's serum, but this is not definitive, since antibody from previous infections can persist after cure. Active infection is demonstrated when the serum titer of antibody in a later convalescent sample (after 1 to 3 weeks) is higher than an early acute sample ("paired sera"). This means the diagnosis is often confirmed after the infection has been resolved. This is acceptable for epidemiologic use, but not so useful for individual patient care.

Recent Progress

Viral Infections.—With the IFA test and ELISA, the diagnosis of respiratory viruses can now be made in a few hours after specimen collection (52,123,125,418). This is a great improvement over isolation by tissue culture which takes several days. With appropriate antisera, the following viral antigens can be identified by the IFA test: influenza (types A and B), respiratory syncytial virus (RSV), measles, adenovirus, parainfluenza 1,2,3,4 (125,264). The economy of time permits large numbers of specimens to be processed. ELISA appears to be very useful for detecting several viruses, but additional experience is needed to evaluate it (264).

Even with a rapid serologic diagnosis, the evidence is only indicative because of possible contamination (i.e., if the clinical sample is negative but the culture is positive, contamination of the culture is suggested rather than a positive diagnosis).

influenza.-Because influenza infection may be fatal, and outbreaks occur annually, with major epidemics and pandemics occurring sporadically, the three main types of influenza, with numerous subtypes and strains, are monitored epidemiologically. Precise strain typing is carried out in order to prepare effective vaccines. Virus isolation and identification from tissue culture is available in 48 hours, but the definitive result may take a week or more. HI and CF tests produce a result in 24 hours, but tests of acute and convalescent serum (samples "paired sera") separated by an interval of 2 to 3 weeks provide definitive diagnosis. The neutralization test is useful but expensive and time-consuming. The direct or indirect immunofluorescent technique can be used with cells obtained from the respiratory tract for a rapid diagnosis even when the patient has no symptoms.

Respiratory Syncytial Virus (RSV).–Precise diagnosis of RSV requires culture and isolation of the virus from tissue culture, then identification with an IFA test. CF or neutralization tests can be used for serologic diagnosis in the patient. Completing the positive identification may take up to 2 weeks, though preliminary results can be obtained by early examination of the cell culture.

Fluorescent antibody tests have been developed to permit rapid diagnosis of RSV infections. If the blood sample is transported promptly to the laboratory and processed immediately, the diagnosis can be made in 4 to 6 hours (264).

Parainfluenza. —Precise diagnosis of parainfluenza viruses requires culture and isolation. Hemagglutination tests identify the virus after 5 to *10* days, while immunofluorescence tests can make the diagnosis in 24 to 72 hours. Detection of a rise in serum antibodies in the individual can be made with the HI test or CF titration, but nonspecific responses make serology an unreliable diagnostic tool. The IFA test has been used for rapid diagnosis, but it has not achieved widespread use.

Adenoviruses. -Definitive diagnosis is by culture, isolation, and use of CF, IHA, or neutralization tests. These three tests can be used to establish the diagnosis using paired serum samples from the patient.

Rhinoviruses. —For the most common agent of the common cold, with over 100 serotypes, routine serologic diagnosis is not very practical, and is not routinely available even in developed countries, though the neutralization test can be used. Even tissue culture isolation is often unsuccessful in detecting the infection, because the conditions for successful isolation require special handling (246).

Corona viruses. —Diagnosis of coronavirus by culture isolation is not a routine procedure and needs specific types of culture cells. Serologic diagnosis can be made using the CF test.

Bacterial and Mycoplasmal Infections.—The diagnosis of bacterial agents of respiratory infections has been greatly improved over the last decade. Rapid and accurate diagnosis is now available in hours rather than days through the use of a variety of diagnostic methods to detect intact bacteria, or in some cases soluble antigen, by the COA and LA tests, ELISA, RIA, CIE, and the IFA test (264).

The COA test can be used for detection of bacteria in specimens such as sputum, serum, urine, and cerebrospinal fluid. It can also be used for strain typing of culture isolates. The test is simple, rapid, sensitive, and specific when the antiserum used is of good quality. Each bacterium to be identified needs a specific antiserum reagent with appropriate antibodies.

The LA test has been used to detect ***Streptococcus pneumonia*** and ***Hemophilus influenza*** in body fluids such as serum, cerebrospinal fluid, and urine. False positives result from nonspecific autoagglutinations and from reaction to antigens common to pathogenic and nonpathogenic organisms.

ELISA can be used to detect bacteria in body fluids. It is highly sensitive for detecting *H.* ***influenzae*** type B, as well as pneumococcal antigen. There are two variations: the direct assay uses a specific antibody (against the bacterial antigen) that is labeled with the enzyme. The indirect assay uses unlabeled specific antibody. The antigen-antibody complex is then identified with labeled antibody that binds to it. The indirect method is very sensitive and more useful, because it limits the need to just one labeled antibody.

RIA is extremely sensitive for detecting S. ***pneumoniae*** and ***H. influenza.*** To evaluate the practical use of RIA, however, more experience is needed. CIE has been successfully used to detect pneumococci, streptococci, and H. ***influenza*** in respiratory secretions and body fluids.

The IFA test can be used to identify bacteria but not soluble antigens. It is very sensitive for detecting ***H. influenzae, S. pneumonia,*** and Bordetella ***pertussis.*** The IFA test can also be used to identify various organisms in culture.

Streptococcus pneumoniae. —Direct microscopic examination of sputum can indicate pneumococci, but the predictive value of this test is variable, because several organisms resemble pneumococci, healthy individuals can carry pneumococci, and a sputum negative for pneumococci does not rule out infection. Sputum culture is the standard procedure. Serologic diagnosis is not practical, because antibodies persist for long periods of time.

Efforts to detect antigen in respiratory tract secretions, blood, and urine by CIE (97,345), as well as by the COA and LA tests (342), have been successful.

Streptococcus pyogenes. —Diagnosis is made by isolation of the streptococci from culture of throat samples. Group determination is made with specific antiserum—the Lancefield precipitation test is considered the standard, though direct fluorescent antibody test is also useful. IFA of throat swab isolates can be obtained after 2 to 24 hours of culture. CIE can be used 6 hours after culture. The COA and LA tests have also been used. There are three convenient tests for detection of serum antibodies.

Bordetella pertussis.—Definitive diagnosis is made 2 to 3 days after culturing a throat sample on specific media, by agglutination with specific antiserum or the IFA test. A rapid diagnosis is available with the direct fluorescent antibody test.

Hemophilus influenzae. -After isolation and culture of a clinical sample, diagnosis is made by microscopically detecting a surface change in the bacterium (the Quellung test). Rapid diagnosis of antigen in secretions and body fluids is available by CIE, the LA test, and ELISA (264).

Mycoplasma pneumonia. —Definitive diagnosis is made by culturing a throat sample on appropriate media, with subcultures made weekly for 8 weeks. When colonies appear, they can be identified visually, though IFA staining confirms the diagnosis.

Rapid diagnosis of sputum by CIE has been demonstrated, but more experience is needed to validate this technique (404). Serologic diagnosis of paired sera is done with the CF test. Detection of antibody in one sample is useless for diagnosis, because high titers persist long after initial infection.

Research Needs

There are many procedures for the diagnosis of ARIs, but various constraints reduce their practical use. Direct demonstration of causative agents is only now becoming an option. Serologic diagnosis using paired sera and serologic diagnosis of culture isolates do not provide timely information for the treatment of the individual patient. Requirements for well-equipped and well-supplied laboratory facilities are another constraint that limit the use of diagnostic procedures in tropical countries.

Continued development of the available and promising technologies for the diagnosis of ARIs is needed. With rapid, simple, reliable diagnostic tests, both the needs of the patient and the community could be better met. Ideally, such tests would not require expensive equipment, reagents, or highly trained operators. In some cases, however, if the tests have certain sophisticated requirements, it may be possible to use them in centralized laboratories.

Arboviral and Related Viral Infections

Because of the general lack of effective treatment for arboviral infections, diagnosis is of less importance to individual patients than to the community, where it is critical for recognizing outbreaks and initiating vector control measures.

Conventional Diagnosis

Clinical diagnosis of the diseases produced by the arboviruses recognizes three syndromes: 1) fevers of an undifferentiated type, frequently called “dengue-like,” with or without rash and usually relatively benign; 2) encephalitis, often with a high case fatality rate; and 3) hemorrhagic fevers, also frequently severe and often fatal (418).

Conventional diagnosis of arboviral disease involves isolation of the virus in newborn mice or cell culture, followed by serologic diagnosis using HI, CF, or neutralization tests. The serologic diagnosis itself is quick and straightforward, but successful culture requires appropriate laboratory equipment, tissue culture materials, and time (usually at least 2 days). More sensitive cell cultures have become available using mosquito cell lines from which early detection can be made using the IFA test (340). It is also possible to inoculate clinical samples into live mosquitoes and then identify the virus using an IFA test of the salivary glands. This technique is very sensitive, but slow (10 to 14 days) (190). Other new methods, such as the RPHA test, are being developed to detect the virus earlier in the culture cycle.

Serologic diagnosis with paired sera still uses conventional methods (HI, CF, and neutralization tests). Single radial hemolysis has been introduced with some important advantages (124). It is as sensitive as the HI test for dengue fever, tick-borne encephalitis, yellow fever, West Nile fever, and Venezuelan equine encephalitis, yet simpler to perform. The method looks promising but needs further evaluation with different viruses.

Direct detection of virus from the patient is dependent on sufficient virus in specimens. The IFA. test has been used with some success for a few diseases (Japanese B encephalitis, Colorado tick fever, Rift Valley fever). ELISA and RPHA methods are being evaluated for detection of viral antigen in body fluids and respiratory secretions. CIE has been used to detect dengue virus in sera from patients with acute diseases but the test has low sensitivity. In all cases of individual direct diagnosis, a negative result is not conclusive.

Detection of specific IgM antibody (which is an early immune response in the acute phase of infection) is carried out by the HI test, ELISA, and the IFA test. This is used for the diagnosis in convalescent patients (when virus has disappeared) and for primary dengue virus.

Recent Progress

MAbs have been developed for certain of the Togaviruses, Bunyaviruses, and Arenaviruses, and nucleic acid hybridization techniques are in various stages of development for some of these viruses. ELISAS are also being developed for members of each group and field tests for some have begun. MAbs to antigens common to groups of viruses have been developed which allow "generic" diagnosis of disease, frequently sufficient to initiate medical therapy and epidemic prevention measures (197).

MAbs for early type-specific identification of the four main serotypes of dengue viruses have been developed by the U.S. Army at the Walter Reed Army Institute of Research (197) and are now generally available. However, dengue virus isolation is still difficult as most patients have low virus concentrations, and the viruses grow poorly in cell cultures. The U.S. Public Health Service laboratory in Fort Collins, CO, also is producing dengue monoclonals in collaboration with WHO. MAbs have also been made to specific surface antigens of several viruses, and ELISA tests based on these reagents are being evaluated (16).

Research Needs

Development of synthetic peptides following sequence analyses of the alphaviruses and flaviviruses could be very important for developing diagnostic reagents to detect serum antibodies (as well as for vaccine development). With regard to the needs of individual patients, rapid diagnostic methods will become increasingly important as drug treatments for arboviruses are developed.

SUMMARY

There is great variability in the availability of diagnostic technologies for diseases of importance in developing countries. In general, however, lack of effective diagnosis is a major obstacle to health care only when health care systems are adequate to act on diagnoses. This situation is not the norm today. While diagnosis is an integral part of medical care, in some cases, diagnostic technologies have even greater value in providing information about the incidence, prevalence, and natural history of diseases of importance to developing countries. Development and use of diagnostic technologies in research could lead to effective integrated disease control strategies.