

6. Newer Diagnostic Techniques and Their Application to Sexually Transmitted Diseases

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Introduction

The diagnosis of a sexually transmitted disease often is more complicated and expensive than treatment of the disease. This is especially true in developing countries where economic resources and access to laboratory diagnostics are limited. Recognition of this problem has led the Rockefeller Foundation to offer a \$1,000,000 prize for the development of affordable STD diagnostics that may be used in developing countries.

Clinical symptoms may be common to several different conditions and physical examination, when possible, is only somewhat more helpful. In the field, the best defined clinical algorithms offer specificity and sensitivity no greater than 50 percent. Laboratory examination remains the definitive method of establishing a diagnosis. Microscopic examination of vaginal and urethral discharges and urinary sediment may aid in defining infections. Although bacterial culture is the current “gold standard” of diagnosis, difficulties in sample handling and inadequacy of laboratory facilities may limit its utility in field settings. The diagnosis of chlamydia by culture requires sophisticated laboratory techniques and is therefore particularly problematic in developing countries.

A number of techniques, sophisticated in design but simple in practice, are becoming available for the detection of causative agents of sexually transmitted diseases. The choice of assay may depend in part on the questions being asked. For example, diagnosing and treating individual patients require assays that provide immediate answers, while determining prevalence of infection requires an assay that may be performed at a single laboratory site on preserved specimens collected from multiple locations. In each case, a different assay may be most cost-effective.

Techniques

Sample Collection

Standard culture techniques for many STDs require obtaining urethral, vaginal or cervical samples. This involves invasive physical examination techniques. More sensitive antigen capture and DNA techniques may be performed using urine samples. Another advance is the detection of antibodies in saliva and other mucosal sites, eliminating the need for venipuncture and serum handling.

Serological Assays

Assays based upon the detection of antibody to infecting organisms are best used as epidemiological tools or when there is no other alternative (e.g., for HIV and syphilis). Antibodies do not develop immediately upon infection. In addition, for many agents the presence of antibody indicates only that infection has occurred sometime previously, but is not necessarily indicative of active infection.

Antigenic crossreactions often have led to false-positive results. For example, antibodies arising in response to infection with many different *Haemophilus* species crossreacted with antigenic preparations of *H. ducreyi*.

Protein blotting techniques have identified crossreactive antigens and newer assays based on recombinant proteins avoid these antigens.

Antigen Capture Methodology

Monoclonal antibodies adhered to a solid matrix ("dipstick") may be used to capture bacterial or viral antigens. The captured antigens are then detected with a second antibody to a different region of the antigen molecule. This antibody usually is labeled with an enzyme that allows for the colorimetric determination of its presence. Combined into diagnostic kits, these antigen capture assays allow for the rapid (i.e., less than 1 hour) identification of infectious organisms. These kits do not require a clinical laboratory.

Clinical tests on urethral swabs and urinary sediment for gonococci or chlamydia demonstrate sensitivities of 80 to 95 percent and specificities close to 100 percent. Under proper storage conditions (refrigeration), bacterial antigens have been found to be stable for more than 1 week.

DNA Hybridization

These assays use labeled DNA probes containing unique gene sequences derived from the organism for which one is testing. These probes will bind specifically to bacterial genetic material in test specimens. If DNA from the organism is present, the test will be positive. These assays have been incorporated into diagnostic kits and can be used in the field.

Extensive testing for the detection of gonococcal infection found these assays to be superior to culture for the diagnosis of infection. That is, cases were found where the DNA assay was positive while the culture was negative. Subsequent investigation determined that these were in fact true positives. Moreover, bacterial DNA has been found to be cleared rapidly following infection, so that persistence of DNA does not appear to be a problem.

DNA Amplification

These extremely sensitive assays use amplification techniques such as the polymerase chain reaction (PCR) and ligase chain reaction (LCR). Using oligonucleotide probes that hybridize specifically to microbial genes, the specimen is subjected to repetitive rounds of DNA replication, so that the quantity of the genetic material is increased several thousand-fold. The amplified gene is then detected with a specific DNA probe. Such assays require moderately sophisticated laboratories.

The extraordinary sensitivity of these assays can create technical and interpretive problems. Infinitesimal levels of contamination can lead to false positives. Or they may reveal true positives but the organisms are so infrequent that the clinical importance of the observation is open to interpretation.

Summary and Conclusions

Highly specific and sensitive assay techniques that can detect the presence of microbes in readily accessible specimens have been developed. These assays can be performed while the patient awaits the results. Because they have been designed for use in industrialized nations, the costs are prohibitive for developing countries. These technologies may be used, however, to define the national or regional prevalence of organisms. Diagnostic algorithms based on the physical signs and symptoms of infections and treatment regimens may then be developed specifically for the prevalent organisms.

Much of the development in STD diagnostics is aiming at tests that are highly sensitive and specific. Such tests are generally expensive and require sophisticated instrumentation. Tests with lower sensitivity and specificity may be more applicable in low resource settings if they are inexpensive and require only very simple technologies.