THE YALE JOURNAL OF BIOLOGY AND MEDICINE 58 (1985), 421–424

Rapid Methods for the Immunodiagnosis of Infectious Diseases: Recent Developments

JOHN E. HERRMANN, Ph.D.,[a] ALAN S. ARMSTRONG, Ph.D.,[b] AND STEPHEN C. EDBERG, Ph.D.[c]

[a]*Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts;* [b]*Diagnostics Division, Abbott Laboratories, North Chicago, Illinois;* [c]*Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut*

Received June 25, 1985

Current techniques for rapid diagnosis of microbial infections by direct detection of the microbial agent are compared. The techniques include enzyme immunoassay (EIA) tests, immunofluorescence, latex agglutination assays, and nucleic acid hybridization procedures. It is concluded that, for the near future, the preferred methods for rapid diagnosis will be by (*1*) EIA tests utilizing monoclonal antibodies and improved enzyme detection systems, and (*2*) improved latex agglutination procedures for certain antigens. Nucleic acid hybridization techniques, as currently performed, will need to be substantially improved to become the methods of choice.

The need for rapid diagnosis of microbial infections in clinical situations is well recognized, in that it allows for prompt, appropriate treatment and the institution of infection control measures in hospital environments. Diagnosis of infections by culture of the infectious agent is the standard by which most rapid methods are compared, but in some cases the agent is either difficult to isolate or has not been cultivated. For these agents, such as hepatitis viruses, Norwalk virus, rotavirus, and human papilloma virus, immunochemical tests are the methods of choice. Even for those agents which can be readily isolated in culture, such as *Chlamydia trachomatis* and many viruses, the time required to obtain results is long, the costs are high, and a high degree of expertise is sometimes required. Thus, rapid and inexpensive methods for detecting these agents are desirable as well.

The major emphasis in rapid methods has been for enzyme immunoassays (EIA) to detect antigen. In fact, there have been descriptions of EIA tests for detection of antigen in clinical specimens for 13 different bacterial species and 13 different viruses [1]. Only a few of these, however, have been utilized for routine diagnosis, and at present there are commercially available antigen EIA diagnostic tests for only six agents: hepatitis B virus, herpes simplex virus, rotavirus, respiratory syncytial virus, *Chlamydia trachomatis,* and *Neisseria gonorrhoeae.* The major reason why more EIA tests are not available is the difficulty in obtaining the sensitivity and specificity that culture provides. Potential means to improve performance of the assays is discussed by Yolken [2]. These include use of monoclonal antibodies, improved antibody conjugation methods, increased magnification of the antigen-antibody interaction by means of

Presented in part at a symposium on "Rapid Methods for Immunodiagnosis of Infectious Diseases," at the annual meeting of the American Society for Microbiology, Las Vegas, Nevada, March 5, 1985

Address reprint requests to: Dr. John E. Herrmann, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, MA 01605

sensitive indicator systems, and use of nucleic acid hybridization techniques. Although nucleic acid hybridization techniques have not yet been shown to be either rapid or highly sensitive for detecting microbial agents in clinical specimens, monoclonal antibodies and amplified indicator systems are becoming effective. Monoclonal antibodies have been shown to provide both higher sensitivity and higher specificity for detecting rotavirus than commercially available polyclonal tests [3], and, based on the limited data available, the use of an amplified enzyme detector system in combination with monoclonal antibodies gives both higher sensitivity and higher specificity than polyclonal antibody tests for detecting *C. trachomatis* genital infection [4].

Monoclonal antibodies are also being used in latex agglutination assays and have been found to be sufficiently sensitive for detection of group B streptococcal antigen in cerebrospinal fluid and concentrated urine specimens [5]. As discussed by Tilton [6], latex agglutination can also be effectively used for detection of *Clostridium difficile* toxins with monoclonal antibodies. Tilton also discusses the chemistry of polystyrene particles, used in latex suspensions, and discusses newer techniques, such as liposome-assisted latex agglutination, which offer the possibility of enhanced sensitivity for latex-based agglutination tests.

One of the major areas in infectious disease targeted for rapid diagnosis is sexually transmitted disease (STD). The need for rapid diagnosis of STD is to provide for prompt, appropriate treatment and to eliminate some of the problems encountered in culture techniques, such as loss of viability in transport and contamination of cultures, especially cell cultures. Also, for diagnosis of chlamydial infections the costs are high and the culture procedures are more complex than those for herpes simplex or *N. gonorrhoeae* isolation. The use of EIA also permits samples to be tested in automated or semi-automated formats. One limitation of the currently available antigen detection tests, whether EIA or fluorescent antibody, is that neither is rapid enough to allow for on-site testing of specimens and same-day treatment of patients. Another drawback, as discussed by Schachter [7], is that the specificity of the EIA tests, although satisfactory for STD clinics, is too low for screening low-risk females. The findings reported by Schachter [7] for an *N. gonorrhoeae* EIA (Gonozyme, Abbott Laboratories) in females showed the EIA to be 87 percent sensitive and 98 percent specific, which is approximately that reported by others. The use of first-voided urine provides a simple, non-invasive method for obtaining samples from males for isolation of *N. gonorrhoeae*. The use of EIA for testing of these samples has been found to be a reliable alternative to culture [8], which was confirmed by Schachter [7]. In addition, the use of EIA has been shown to be superior to culture in situations requiring transport of specimens [9].

Rapid methods for diagnosing *C. trachomatis* infection by EIA (Chlamydiazyme, Abbott Laboratories; IDEIA, Boots-Celltech) and by direct fluorescent antibody (FA) tests (Microtrak, Syva) are also available. The sensitivity of Chlamydiazyme has been reported to be from 72 percent to 83 percent [10–12] and the specificity from 94 percent to 98 percent [10–12]. The sensitivity and specificity of the IDEIA chlamydial EIA, which utilizes monoclonal antibodies, have been reported to be 95.5 percent and 99 percent, respectively [3]. The polyclonal EIA tests for chlamydial antigens have the same limitation as the gonococcal EIA tests, that of a low predictive value for positive tests in low-risk populations [11]. It has been found, however, for both the gonococcal and chlamydial EIA tests that the majority of false-positive results occur at or near the cut-off point selected for the assay [12,13]. Thus, if these data are provided by the

manufacturer of the tests, users should be able to better interpret the reliability of positive test values obtained in screening low-risk populations.

The sensitivity of the direct FA technique for diagnosing *C. trachomatis* genital infection was found by Schachter [7] to be only 70 percent, which is lower than most reports. Those tested were asymptomatic individuals, which may have been the major reason for the low sensitivity obtained. Diagnosis of herpes simplex infection by direct FA and EIA is also possible with commercially available reagents. The sensitivity of one EIA (Ortho Diagnostics) has been reported to be only 53 percent and 72 percent [14,15], which makes this test of limited value. The results do indicate, however, that EIA is a feasible approach and with further refinements could be used as an alternative to culture.

The use of nucleic acid hybridization techniques has also been described for diagnosis of *N. gonorrhoeae* [16], herpes simplex [17], and *C. trachomatis* [18,19]. None, however, has been shown to be higher in sensitivity than EIA for the same agents, and for *C. trachomatis,* specificity may also be a problem [19]. The time required to obtain results is also longer, and most of the assays use radioactive probes. These and other factors in nucleic acid probe technology are discussed in the accompanying article by Edberg [20]. For probes to become effective for routine clinical laboratory use, Edberg emphasizes that (*1*) radioactive markers must be replaced with non-radioactive ones; (*2*) the sensitivity needs to be improved, up to 100-fold; (*3*) the probes must be broadly reactive enough to detect all members of a given species; and (*4*) the techniques used need to be simplified and be adaptable to automation.

In summary, the near future of immunodiagnostic assays should be improved EIA and latex agglutination tests, followed by application of probe technology. Although many of the current EIA tests for microbial antigens have not realized their potential, there are reasons to believe this situation will improve. For detection of antigen, which offers a rapid and direct means of diagnosing microbial infections, the major problem has been lack of sensitivity. Increasing the sensitivity of polyclonal EIA tests by using more concentrated immunoreagents or more sensitive enzyme substrates has often resulted in a loss of specificity. From the reports available to date, it appears that use of the appropriate monoclonal antibodies may solve the problem of sensitivity for detecting many infectious agents in clinical samples. With the increasing number of monoclonal antibodies available for various microbial antigens, we can expect that more of them will be utilized for EIA detection systems. If monoclonal antibodies of high affinity can be obtained, the EIA tests should be sufficiently sensitive for detecting most microbial agents in clinical samples.

A similar situation exists with nucleic acid hybridization techniques in regard to sensitivity. Current tests require the same or higher number of infectious units than are required for detection by EIA. These tests will also need to be simplified and be able to provide same-day diagnosis to be practical diagnostic reagents.

REFERENCES

1. Herrmann JE: Enzyme-linked immunoassays for the detection of microbial antigens and their antibodies. In Advances in Applied Microbiology, Volume 31. Edited by AI Laskin. Orlando, Academic Press, Inc, in press
2. Yolken RH: Enzyme immunoassay for the detection of microbial antigens and prospects for improved assays. Yale J Biol Med, in press

3. Herrmann JE, Blacklow NR, Perron DM, Cukor G, Krause PJ, Hyams JS, Barrett HJ, Ogra PL: Monoclonal antibody enzyme immunoassays for the detection of rotavirus in stool specimens. J Infect Dis 152:830–832, 1985
4. Caul EO, Paul ID: Monoclonal antibody based ELISA for detecting *Chlamydia trachomatis*. Lancet i:279, 1985
5. Rench MA, Metzger TG, Baker CJ: Detection of group B streptococcal antigen in body fluids by a latex coupled monoclonal antibody assay. J Clin Microbiol 20:852–854, 1984
6. Tilton RC: Diagnosis of microbial infections by latex agglutination procedures. Yale J Biol Med, in press
7. Schachter J: Immunodiagnosis of sexually transmitted disease. Yale J Biol Med 58:443–452, 1985
8. Rudrik JT, Waller JM, Britt EM: Efficacy of an enzyme immunoassay with uncentrifuged first-voided urine for detection of gonorrhea in males. J Clin Microbiol 20:577–578, 1984
9. Martin R, Wentworth BB, Coopes S, Larson EH: Comparison of Transgrow and Gonozyme for the detection of *Neisseria gonorrhoeae* in mailed specimens. J Clin Microbiol 19:893–895, 1984
10. Amortegui AJ, Meyer MP: A nonculture test for identification of *Chlamydia trachomatis*. J Reprod Med 30(Suppl):279–283, 1985
11. Herrmann JE, Howard LV, Leman C: Antigen detection by enzyme immunoassay (Chlamydiazyme) as a screening method for detecting *Chlamydia trachomatis* genital infection in various populations. In Medical Virology III. Edited by L de la Maza, EM Peterson. New York, Elsevier Science Publishing Co, 1984, p 366
12. Jones MF, Smith FT, Houghlum AJ, Herrmann JE: Detection of *Chlamydia trachomatis* in genital specimens by the Chlamydiazyme test. J Clin Microbiol 20:465–467, 1984
13. Bronken TP, Dyke JW, Andruszewski MH: A solid-phase enzyme immunoassay in detection of cervical gonorrhea in a low-prevalence population. J Family Pract 20:43–48, 1985
14. Warford AL, Levy RA, Rekrut KA: Evaluation of a commercial enzyme-linked immunoassay for detection of herpes simplex virus antigen. J Clin Microbiol 20:490–493, 1984
15. Morgan MA, Smith TF: Evaluation of an enzyme-linked immunosorbent assay for the detection of herpes simplex virus antigen. J Clin Microbiol 19:730–732, 1984
16. Totten PA, Holmes KK, Handsfield HH, Knapp JS, Perine PL, Falkow S: DNA hybridization technique for the detection of *Neisseria gonorrhoeae* in men with urethritis. J Infect Dis 148:462–471, 1983
17. Redfield DC, Richman DD, Albanil S, Oxman MN, Wahl GM: Detection of herpes simplex virus in clinical specimens by DNA hybridization. Diagn Microbiol Infect Dis 1:117–128, 1983
18. Palva A, Jousimies-Somer H, Saikku P, Vaananen P, Soderlund H, Ranki M: Detection of *Chlamydia trachomatis* by nucleic acid sandwich hybridization. FEMS Microbiol Lett 23:83–89, 1984
19. Hyypia T, Jalava A, Larsen S, Terho P, Hukkanen V: Detection of *Chlamydia trachomatis* in clinical specimens by nucleic acid spot hybridization. J Gen Microbiol 131:975–978, 1985
20. Edberg SC: Principles of nucleic acid hybridization and comparison with monoclonal antibody technology for the diagnosis of infectious diseases. Yale J Biol Med 58:425–442, 1985