MEDICAL VIROLOGY IV

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FOREWORD

The awareness of the medical community of the key role played by viruses on the overall welfare of the individual and the society at large continues to increase. As a result, in the area of diagnostic virology there is a growing number of research institutions and profit organizations that are becoming involved in the development of laboratory tests for viral infections. Methods and approaches that were research tools not too long ago are today applied at the clinical bench. The utilization of antiviral drugs and genetic engineering in the treatment and prevention of viral infections is no longer a science fiction dream for the future but a very realistic possibility for the present. The practicing physician is now faced with a pleiad of patients at a high risk of acquiring viral infections that, for proper management, require a basic understanding of the mechanisms involved in viral pathogenicity. The public at large is gaining a knowledge of health related problems and, as a result, demands better and more sophisticated delivery of services in the area of medical virology. In addition, governments as providers of research resources and medical care now play a fundamental role in the development of medical virology.

In this book, we want to bring to our readers some of this new knowledge and excitement that all the participants shared during the 1984 International Symposium on Medical Virology.

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Irvine, California, February, 1985

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Hypothetical scheme for multiple cycle evolution of influenza variants involving "rescue" of intermediate defective mutants by phenotypic mixing. (Because of the segmented nature of the influenza genome mutations in the HA gene will not affect transcription or translation of other viral genes.)

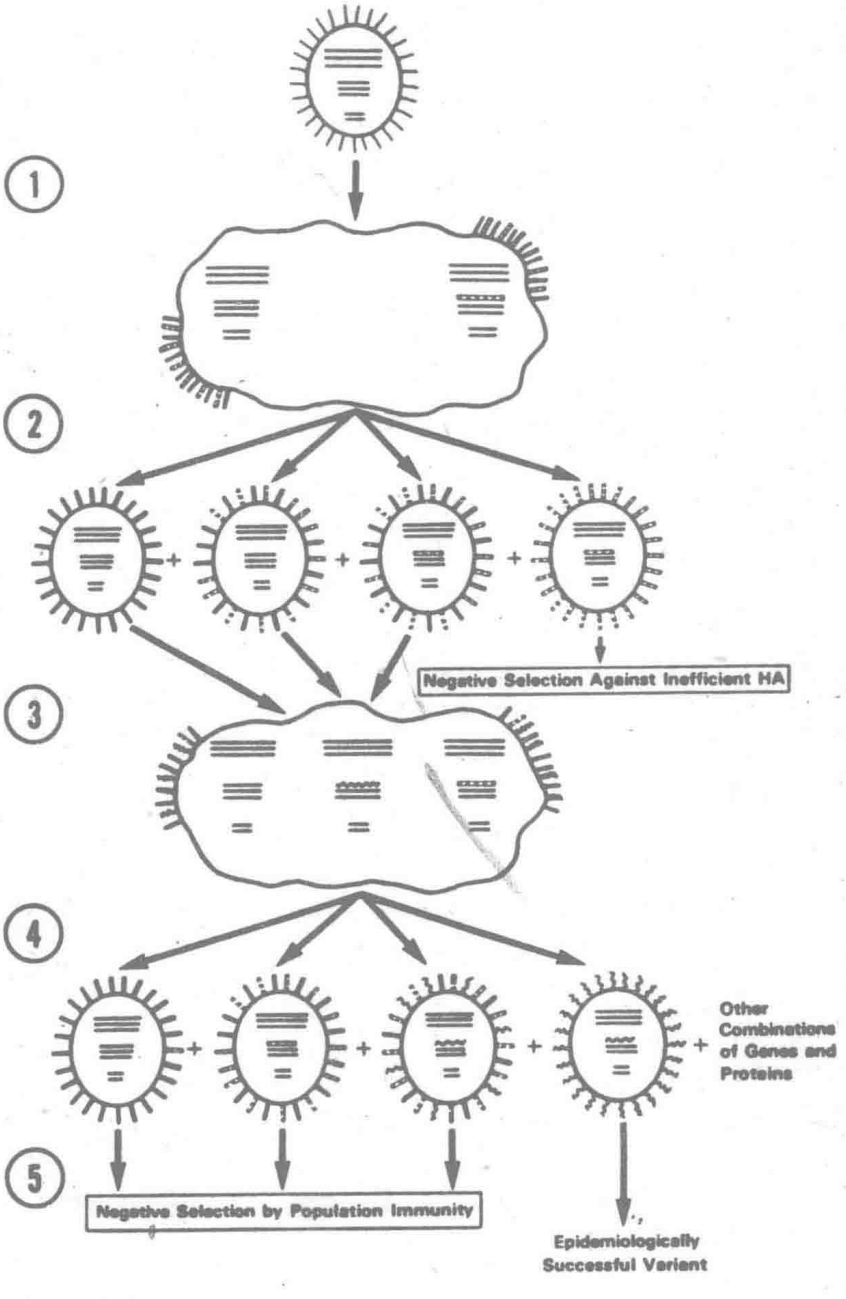

Figure 2. Step 1. Mutant gene evolves in infected cell: the mutant HA product of this gene has reduced biological activity; Step 2. The mutant HA gene is "rescued" by packaging in virus particles with some normal HA proteins; Step 3. Mixture of virus particles containing normal and mutant HA is transmitted to next case. During replication, a second mutation appears in the HA gene that restores biological activity; Step 4. A new mixture of virus phenotypes and genotypes is produced; Step 5. The virus containing the appropriate combination of mutations in its HA has higher transmissibility than the original virus or intermediate mutant and becomes the next epidemic variant.

CONTENTS

APPLICATION OF RAPID TECHNIQUES IN THE CLINICAL VIROLOGY LABORATORY

Harvey M. Friedman

INTRODUCTION

Diagnostic virology has gradually assumed an increasingly important role in the medical community. The ability to establish a definitive diagnosis of a viral infection within 1-5 hours of receiving a sample has greatly enhanced the image of the viral laboratory and has expanded the impact the laboratory can have on clinical decision making. Aspects of decision making influenced by rapid diagnosis of a viral infection include avoiding unnecessary diagnostic testing, eliminating unneeded medications and, in some cases, permitting earlier discharge from the hospital. In a few instances, rapid diagnosis also helps guide the use of antiviral drugs, especially in the treatment of herpes simplex virus (HSV) or varicella zoster virus (VZV) infections.

Three general approaches can be used to establish a rapid viral diagnosis. The most widely used strategy is to attempt identification of viral antigens in specimens immediately after collection, without waiting for the virus to grow in cell culture systems. A second approach is to identify viral antigens or nucleic acids in tissue culture cells before cytopathology (CPE) develops. A third method is to detect viral specific IgM antibodies in serum taken early in the course of infection. The main focus of this report will be on the first method, that is, direct antigen detection in clinical specimens.

For widespread application of rapid viral techniques in clinical laboratories, excellent reagents must be available from commercial sources. In recent years, we have seen major progress in this area. However, even with excellent reagents, there are limitations to the sensitivity of rapid techniques, the most important of which is the amount of viral antigen produced during infection. In the first few days of

the clinical illness, maximal amounts of virus are likely to be produced, which emphasizes the importance of obtaining specimens early in the disease. Laboratories performing direct antigen detection tests should correlate their rapid results with more conventional diagnostic methods, generally with viral isolation. This serves as a form of internal quality control. In some circumstances, when sufficient samples have been tested by both rapid and standard methods, it may be possible to omit the standard assay. In general, this should only be done if the sensitivity and specificity of the rapid method are equivalent to those of the standard assay.

VIRUSES OF IMPORTANCE TO THE CLINICAL LABORATORY

The diagnostic virology laboratory at The Children's Hospital of Philadelphia is oriented predominantly towards an in-patient population. The laboratory mainly services two hospitals, the 250-bed hospital, The Children's Hospital of Philadelphia and the adjacent 680-bed hospital, The Hospital of the University of Pennsylvania. During 1983, the laboratory performed 13,755 tests, including 4241 viral isolations, 4837 serologies; 3613 hepatitis assays, 664 chlamydia cultures, and 400 rapid tests. Seven hundred and nineteen of the 4241 cultures (17%) grew a virus. Table 1 lists the types of isolates obtained. In addition to those viruses shown, rotavirus was detected by electron microscopy in 28 stool specimens.

From these results, it is apparent that the emphasis for rapid diagnosis can be placed on relatively few viruses. The agents chosen for discussion in this review include HSV, cytomegalovirus (CMV), VZV, respiratory syncytial virus (RSV), rotavirus and Chlamydia trachomatis. Some of these, as noted in Table 1, are of obvious importance to the diagnostic laboratory including HSV, CMV, and RSV. Others merit discussion because recent advances in technology will likely permit rapid and accurate diagnosis using commercially available reagents.

Respiratory Syncytial Virus

Philip Gardner, from Newcastle upon Tyne, England, was one of the pioneers in the field of rapid viral diagnosis. Gardner and McQuillin (1968) used immunofluorescent (IF) techniques to directly stain nasopharyngeal secretions and determined the cause of some acute respiratory

TABLE 1. VIRUSES ISOLATED AT THE CHILDREN'S HOSPITAL OF PHILADELPHIA VIROLOGY LABORATORY IN 1983

Virus	No. of Isolates
HSV	299
CMV	132
RSV	98
Adenovirus	64
Enterovirus/Rhinovirus	78
Parainfluenza 1-3	21
Influenza A	12
Influenza B	2
VZV	12
Rubella	1
Total	719

infections on the day of admission. Nasopharyngeal secretions were examined for RSV by IF in children with bronchiolitis, pneumonia, bronchitis, croup and whooping-cough. Of 32 samples, 19 were positive for RSV by culture and 17 of these were also positive by IF. All 15 culture negative samples were negative by IF. Since these excellent early results, Gardner and McQuillin have extended the scope of antigens examined by IF in respiratory secretions and have also greatly increased the number of samples studied for rapid diagnosis of RSV (see review in Gardner, 1980).

Kaul et al. (1978) were among the first in North America to report on the use of rapid IF techniques for diagnosis of RSV. These investigators found excellent correlation between rapid IF and viral isolation. Of 387 samples tested, discrepancies were note in 22, 17 of which were IF positive but culture negative, and five of which were IF negative but culture positive. As discussed in a later section of this review, it is likely that many of the IF positive but culture negative samples were true positives.

In recent years, the availability of excellent polyclonal antiserum for rapid IF diagnosis of RSV (Burroughs-Wellcome Co., Research Triangle Park, NC) has permitted wider use of this assay. However, the applica-

tion of monoclonal antibody technology to the field of rapid viral diagnosis offers an alternate source of antibodies. Bell et al. (1983) compared viral culture with rapid IF using either bovine polyclonal or mouse monoclonal antisera. They found an excellent correlation among the three methods. The monoclonal antibody tended to produce less nonspecific background fluorescence than the polyclonal serum. The investigators thought that less experienced workers may find the monoclonal assay easier to interpret. Similarly, Kim et al. (1983) found an excellent correlation between viral culture and rapid detection using monoclonal antibodies in an indirect immunofluorescence (IFA) assay.

Cevenini et al. (1983) evaluated immunoperoxidase (IP) staining for rapid RSV diagnosis and compared it with IF. The investigators used commercially available anti-RSV serum and conjugates. Results were identical by the two assays. A potential advantage for the IP technique is that it does not require the expensive equipment needed for IF.

Chao et al. (1979) evaluated an enzyme immunoassay (EIA) for rapid diagnosis of RSV. The investigators used an antibody capture assay to detect RSV antigen in nasal secretions from infants with respiratory disease. RSV was detected in 23 of 29 specimens positive for virus by tissue culture and in one of 36 samples negative by culture. The investigators used a blocking assay to confirm positive EIA results, which indicated that the false-positive result was likely a true positive. EIA was also compared with direct staining of secretions by IF. Discordant results occurred in 10 of 81 specimens. Seven of 10 were EIA positive but fluorescence negative, while three were fluorescent positive and EIA negative. Overall, the EIA was found to be somewhat more sensitive than fluorescence and was suggested as an alternate approach for rapid diagnosis.

To summarize the RSV results, it is clear that rapid diagnosis of this common childhood respiratory illness is now possible for the clinical laboratory. A major, and as yet unresolved issue, is whether rapid test results need to be confirmed by viral isolation. As laboratories begin to perform the various rapid assays for RSV, it seems very important that the initial evaluation include comparisons between rapid results and viral isolation. As laboratories become more experienced with the rapid tests, they may decide to avoid duplication of results by omitting viral cultures on those samples which are clearly positive in the rapid assay.

Herpes Simplex Virus

Rapid diagnosis of HSV is particularly important in patients with encephalitis, in neonates with suspected generalized HSV and in immunocompromised hosts with atypical-appearing muco-cutaneous rashes. Each of these infections can be treated with antiviral drugs; therefore, an accurate and rapid diagnosis is important to guide therapy.

Tzanck (1947) was perhaps the first to describe a rapid method for cytologic evaluation of vesicular lesions. Recently, Solomon et al. (1984) performed a study to assess the accuracy of the Tzanck preparation compared with viral culture. Patients included those with primary HSV (13 cases) and recurrent infections (17 cases). The lesions were oral in 16 patients, genital in 11 and elsewhere on the face or hand in three. Thirty-two samples were taken from 30 patients. For the Tzanck stain, the lesion base was scraped with a scalpel blade which was then touched to a glass slide. The slide was air-dried and stained for 15 seconds with toluidine blue. Twenty-five of 32 (78%) samples were culture positive, while 17 (53%) were Tzanck positive. When results were evaluated according to the stage of the lesion, the investigators found that during the vesicular stage all 15 samples were positive by viral culture while 10/15 (67%) were positive by Tzanck smears. During the pustular stage, 8/11 (73%) were culture positive while 6/11 (55%) were Tzanck positive. At later stages (crusting or ulcerating), 2/6 (33%) were culture positive compared with 1/6 (17%) by Tzanck smear. Overall, 68% of HSV positive by culture were also positive by Tzanck smear. Therefore, this rapid and inexpensive test was useful to confirm a suspected HSV infection but was not very sensitive. It is important to note that a positive Tzanck smear does not distinguish between HSV and VZV since both produce multinucleated giant cells in which the nuclei have a "ground-glass" appearance. Intra-nuclear inclusions are also seen during infection with both viruses.

Goldstein et al. (1983) evaluated monoclonal antibodies directed against type-specific proteins on HSV-1 or HSV-2 for rapid diagnosis. Fifty-nine patients with oral, genital, ocular and mucocutaneous HSV infection and 43 throat or genital swabs from healthy controls were examined. One hundred and six samples from these patients were compared by rapid IF and viral culture. All 43 specimens from controls were negative by rapid IF and viral culture. Of the 63 specimens from patients with a clinical diagnosis of HSV, 54 (86%) were positive by each method.

In six cases the culture was positive but the rapid test was negative; however, in an additional six cases the rapid test was positive while the culture was negative. These latter samples can be considered as true positives by IF since they were taken from patients who had HSV disease based on clinical criteria. Of interest, the investigators used three different anti-HSV-2 monoclonal antibodies in the rapid assay. Thirty-four samples were positive with at least one of the HSV-2 antibodies; however, in only 23 specimens did all three antibodies stain positive, in 10 samples only two of the antibodies were positive and in one specimen only a single monoclonal antibody was positive. When these same antibodies were used to type HSV isolated in culture, all three antibodies detected all HSV-2 isolates. This indicates that for direct antigen detection a combination of several monoclonal antibodies may offer an advantage over a single antibody preparation.

An important aspect of rapid testing by IF or IP staining is to determine whether an adequate sample has been collected. Goldstein et al. (1983) considered 20 cells per slide as adequate. Although this is arbitrary, it does point out an important advantage of IF or IP microscopy over enzyme or radioimmunoassays (RIA). The former procedures, which use microscopy, permit the observer to determine whether an adequate sample has been submitted.

Moseley et al. (1981) used commercially available polyclonal antibodies to compare viral isolation, direct IF and indirect IP techniques for detection of genital HSV. When specimens were collected during the vesicular stage of the eruption, isolation was positive in 93% and the direct assays in 77%. However, when specimens were taken later in the illness, the isolation rate declined (83% of pustules and 72% of ulcers were positive) as did the frequency of positive results by the direct assays. Overall, the IP assay correlated better with viral isolation than did IF.

Schmidt et al. (1983) performed a similar study comparing direct IF, direct IP staining and viral culture. Approximately 10% of the samples submitted for direct testing contained fewer than 5-10 epithelial cells on the slide and were considered inadequate for rapid testing. Approximately half of the inadequate samples were positive for HSV by viral isolation. These isolates would likely have been missed had the rapid assay been the only test performed. Both IP and IF were specific in that there were very few false positive results; however, both assays