Diagnostic Virology in a Community Hospital

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INTRODUCTION

Seven and one-half years of experience in a small diagnostic virology laboratory of a large inner-city hospital are reported. Seven hundred fifty-one viruses were isolated from over 8,000 specimens, using two types of tissue culture cells, human and monkey kidney. The most common isolates were Herpes simplex viruses (HSV) and Enteroviruses. Similar results have been reported by larger laboratories. Sensitivity for HSV in monkey kidney cells was only 75 percent that in human cells. An enzyme-linked immunosorbent assay (ELISA) for cytomegalovirus (CMV) was found to be a suitable substitute for the traditional complement fixation test (CF). IgM antibodies were not found in all HSV infections, but these antibodies did appear before CF antibodies in some cases. Monoclonal antibodies to HSV were effective in typing isolates, but for detection of viral antigen in brain smears of HSV encephalitis patients, polyclonal antibody gave better results.

In 1976 a small diagnostic virology laboratory was set up at Saint Michael's Medical Center, in Newark, New Jersey, a teaching affiliate of the state medical school, the University of Medicine and Dentistry of New Jersey at Newark. It was developed at the request of Dr. Leon Smith, Director of Infectious Diseases at Saint Michael's, under the auspices of the Infectious Disease Physicians Group of Northern New Jersey. Its purpose was to help the infectious disease physicians manage their patients with viral infections. This is a densely populated urban area of the United States which had previously relied on laboratories in New York City or Philadelphia. Specimens were received from approximately 40 different hospitals in northern New Jersey with a patient population consisting of about 60 percent children, and 40 percent adults. Laboratory personnel included one full-time director and one full-time technician. Because of the limited personnel, it was decided that the most efficient use of facilities would be to offer virus isolation from patient specimens and selected viral serology. Virus identification was by characteristic cytopathic effect (CPE), immunofluorescence (IF), complement fixation (CF), and neutralization testing (NT). Cooperation with the state laboratory at Trenton, New Jersey, was necessary for final enterovirus, influenza, and adenovirus identification.

This paper describes the problems and successes of a small diagnostic virology laboratory, specifically the methods and results of isolation, the limited serology, and selected examples of the type of limited research that could be performed under these conditions.

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The primary objectives of the laboratory were to isolate and identify viruses from clinical specimens. Rather than use the limited time to make our own cell culture tubes, roller tubes from commercial sources were received weekly. Use of one tube of human diploid cell strains, such as WI-38, MRC-5, or Flow 2000, and one tube of primary monkey kidney cells, such as Rhesus or Cynomolgus monkey (containing antibodies to SV-5 and SV-40), was chosen as the most economical, efficient way to isolate the greatest majority of human viruses. Specimens were processed by methods as described by Hsiung [1]. A roller tube of each cell type was inoculated with 0.1–0.3 ml of processed virus specimen and incubated in a stationary fashion at 35°C for a minimum of two weeks. Viruses difficult to isolate such as rubella virus or coxsackie A viruses were sent to reference laboratories. Figures 1 and 2 show the growth of the lab as reflected in the number of specimens received for virus isolation and the number of viruses isolated. It took two years to reach a level of about 1,000 specimens per year, and then another six years to reach the 2,000 mark. The percentage of specimens from which viruses were isolated continued at a 9 to 10 percent level over all the years. Table 1 shows the types of specimens received and the number of virus types isolated from each. When compared to previously published results from two larger laboratories, the California Health Department 1966–1970 [2] and the Mayo Clinic 1962–1971 [3], many of our results were similar. The viruses most frequently isolated in our laboratory were Herpes simplex viruses (HSV) and the Enteroviruses group. This result was similar to that of the Mayo Clinic. However, Enteroviruses were the most frequent isolates at the California Health Department, with HSV third after adenoviruses. The greatest variety of virus types in our laboratory was isolated from throat specimens, as was also seen at both the Mayo Clinic and the California Health Department. In our laboratory specimens with the lowest percentage isolation rate were CSF and urine, while at Mayo Clinic and the California Health Department CSF and autopsy specimens had the lowest rates. At the California Health Depart-

![Number of Specimens for Virus Isolation](image)

**FIG. 1.** Number of specimens received per year for virus isolation from 1976 to 1983.
ment CSF, urine, and autopsy specimens were least likely to show viruses. These results suggest that throat specimens have the best yield in variety and percentage isolation rate. Our overall isolation rate of 9.3 percent was lower than that reported by Schmidt (20 percent) and Hermann (14 percent). This can be partly explained because few specimens were received in our laboratory for isolation of the respiratory viruses, such as influenza, parainfluenza, respiratory syncytial virus,

![Graph](image)

**FIG. 2.** Number of viruses isolated per year at the diagnostic virology laboratory, from 1976 to 1983.

### TABLE 1

| Types of Specimen | Fecal | Throat | CSF | Lesion | Urine | Autopsy | Total |
|--------------------|-------|--------|-----|--------|-------|---------|-------|
| No. specimens examined | 941   | 1,376  | 2,051 | 2,206  | 1,342 | 91      | 8,017 |
| No. viruses isolated: |       |        |      |        |       |         |       |
| Adeno              | 3     | 11     | 0    | 2      | 1     | 0       | 17    |
| Entero             | 54    | 28     | 52   | 6      | 6     | 0       | 146   |
| Flu                | 0     | 7      | 0    | 0      | 0     | 0       | 7     |
| Paraflu            | 0     | 1      | 0    | 0      | 0     | 0       | 1     |
| Mumps              | 1     | 5      | 5    | 0      | 0     | 0       | 11    |
| Measles            | 0     | 1      | 0    | 0      | 0     | 0       | 1     |
| RSV                | 0     | 5      | 0    | 0      | 0     | 0       | 5     |
| HSV                | 7     | 32     | 0    | 467    | 5     | 5       | 516   |
| V-Z                | 0     | 0      | 0    | 20     | 0     | 1       | 21    |
| CVM                | 0     | 4      | 0    | 1      | 19    | 1       | 25    |
| Vac                | 0     | 0      | 0    | 1      | 0     | 0       | 1     |
| Total recovered    | 65    | 94     | 57   | 497    | 31    | 7       | 751   |
| Isolation rate (%) | 6.9   | 6.8    | 2.7  | 22.5   | 2.3   | 7.6     | 9.3   |

*First six months, 1983
adenovirus, and rhinovirus. In the larger laboratories these respiratory isolates helped raise their overall isolation rate. Also in our laboratory, many specimens from material with a low percentage rate of isolation, such as CSF and urine, were received. In addition, since our laboratory was new, few specimens were rejected. Many of the physicians who sent us specimens were using a diagnostic virology laboratory for the first time and were not accustomed to ordering the proper specimens. To help educate physicians to order better specimens an article entitled “Physicians' Guide to Laboratory Diagnosis of Viral Infections” was written for the Journal of the Medical Society of New Jersey [4] and numerous lectures were given at local hospitals. In our small laboratory, and in other large laboratories [5], the greatest challenge was to educate the physicians to order appropriate specimens.

Since specimens were inoculated into both human and monkey cells, there was an opportunity to compare HSV isolation in both cell types, as shown in Fig. 3. Previous reports have stated that HSV type 1 would not grow in primary monkey kidney cells [6]. However, this does not appear to be true, and 212 of 241 HSV isolates shown in Fig. 3 were typed by immunofluorescence, with 89 found to be type 1 and 123 found to be type 2. There was no difference found between type 1 and type 2 with regard to their ability to be isolated in primary monkey kidney cells. However, sensitivity of the monkey cells as compared to human cells was only about 75 percent, and the time required for virus detection was somewhat slower, showing that monkey kidney cells were not the optimum substrate for HSV in a diagnostic virology laboratory.

SELECTED EXAMPLES OF CLINICAL EXPERIENCE WITH NEWER TESTS

Although much of the time in a small diagnostic virology laboratory is needed for routine clinical diagnosis, some research was carried on by comparing newer techniques with the older ones, especially in an attempt to improve serodiagnosis.
Because the standard CF test for CMV was difficult to perform, time-consuming, and very insensitive, a new ELISA kit for CMV was evaluated by comparing results on the same serum specimen with the standard CF test. Figure 4 shows comparative results on 153 clinical serum specimens by the two techniques. On the left is the standardized absorbance at 405 nm and on the right the report to physicians as negative, low, mid, or high positive. Qualitative detection of CMV antibody agreed in 150 of 153 sera tested. Exact quantitative correlation between CF and ELISA tests was not found. There was a great scatter of absorbance values for CF values of 1:16 to 1:128; i.e., sera with similar CF values often showed a wide range of absorbance at 405 nm. These results are similar to what others have reported [7,8,9,10]; one possible reason for these differences is that different types of antibodies were being measured by CF and ELISA. However, our results showed that ELISA was a valid substitute test for the CF test in detection of CMV antibodies, confirming results reported by Kieffer et al. [11].

Because HSV serology results are sometimes difficult to interpret, an HSV-1 and HSV-2 specific IgM indirect immunofluorescent test kit from Microbiological Research Corporation (Bountiful, UT) was compared to the standard CF test for HSV. Figure 5 shows 60 sera from 53 patients with HSV infections demonstrated by virus isolation. These patients were screened for HSV-specific IgM antibody at 1:5 dilution and their CF antibody titers determined by standard CF techniques. More than one serum sample was obtained from three patients. Sera collected within five days of virus isolation were considered acute sera. Sera which were collected five or more days after virus isolation were called convalescent. Since primary or recurrent infection could not be determined in all cases, this is not shown on Fig. 5. Of 22 sera found positive for HSV IgM, eight were from known primary infections as determined by clinical history, one from a recurrent infection, and the rest were of
unknown origin. Of 38 sera found negative for HSV IgM, five were from known primary infection, four were from recurrent infection, and the rest were of unknown origin. Six of the ten sera which did not have a detectable CF titer (< 1:8) were positive for IgM, and two of four negative for IgM were acute sera from primary infections that may have been taken too early during infection for IgM to have developed. Results showed that in a few cases IgM antibody was detectable before CF antibody. Although the numbers are very small, these preliminary data also show that at least in a few cases primary infection did not elicit a detectable IgM response. Much more data will have to be collected to learn the significance of IgM response in primary and recurrent HSV infections.

Typing of HSV isolates was done by direct immunofluorescence using rabbit polyclonal antibody to HSV-1 and HSV-2. Although there was cross-reactivity between type 1 and 2, typing was possible by using dilution techniques. When mouse monoclonal antibody specific for either HSV-1 or HSV-2 became commercially available either from Immunolok (Ortho Diagnostics, Raritan, NJ), using an indirect system, or from Syva (Palo Alto, CA) using a direct system, typing of HSV was then done by the monoclonal antibody. The Immunolok product gave a whole cell fluorescence with type 1 virus, and a nuclear fluorescence with type 2. The Syva material gave a whole cell fluorescence in both. There was no cross-reactivity. In both cases virus typing was easily performed. Other investigators have shown that monoclonal antibodies work well for HSV typing [12,13]. In one recent case of proven HSV encephalitis a direct comparison on infected brain smears was made between polyclonal anti-HSV-1 (M.A. Bioproducts: direct type), Immunolok monoclonal antibodies against HSV type 1 and 2, and Syva monoclonal antibodies against both HSV types. Only polyclonal and monoclonal type 1 antibodies were positive on infected brain cells. Both Immunolok and Syva type 2 were negative, but the polyclonal type 1 gave a much more intense reaction than did either of the monoclonal antibodies and therefore was much easier to detect.
CONCLUSION

A small diagnostic virology laboratory may contribute a large amount of useful information to clinicians. Since every type of virology testing could not be done, the decision was made to concentrate on virus isolation using only two cell lines. These gave results comparable with those reported by much larger laboratories. A large percentage of the isolation results was obtained within five days or less of receipt of specimens, so that patient management decisions could be made in light of this data. Serology has been done only on a limited basis. Since ours is a new laboratory, much effort was spent on physician education to improve the quality of the specimens received. Limited research has shown that new techniques such as ELISA, IgM antibody detection, and use of monoclonal antibodies may help to improve the reliability of diagnostic virology laboratories in the near future.

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