Diagnostic Virology Laboratory
Within a Microbiology Setting

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The virology section at St. Francis Hospital and Medical Center, Hartford, Connecticut, is not a separate laboratory division but is a part of the microbiology division and is supervised by the same personnel who supervise bacteriology, mycology, mycobacteriology, and serology. Current volume is over 1,000 cultures yearly with 12 to 24 percent positive. Isolates are confirmed and typed by the Connecticut State Health Department Laboratory. Specimen distribution, percentage positive specimens, and distribution of viral isolates are similar to those reported from microbiology laboratories with separate virology laboratories directed by a full-time doctoral-level virologist.

Our seven years' experience demonstrates that a microbiology laboratory without a full-time doctoral-level virologist can provide clinically useful virologic information.

In 1976 Herrmann and Herrmann surveyed viral diagnostic facilities available to pediatrics groups in 151 medical centers. Among 115 respondents in the United States almost 60 percent had on-site viral diagnostic laboratories [1]. The survey revealed that physicians were more likely to use the virus laboratory if it was located within their own institution. Reasons for not utilizing the virus laboratory included too much time before a result was available, high cost, un-useful information, and inability to treat virus infections.

Only two community hospitals have described their experience with on-site diagnostic virology. Butterworth Hospital in Grand Rapids, Michigan, in 1972 established a screening program for enterovirus isolation [2]. Cell cultures showing cytopathic effect (CPE) were sent to the Michigan Department of Public Health for virus identification. Thirty-four percent of cultures were positive. They felt confirmation of these cases of enteroviral aseptic meningitis prevented much unnecessary hospitalization.

The Minneapolis Veterans Administration Hospital Laboratory used a different approach [3]. Techniques for primary viral isolation were established for those viruses most frequently isolated in their patient population (herpes viruses). If other viruses were suspected, specimens were sent to a reference laboratory. Parallel testing of 50 duplicate specimens sent to the reference laboratory resulted in equal numbers of viral isolates, but the on-site laboratory reported results an average of 3.2 days earlier.
Our experience using a reference laboratory for virus isolation was poor. From 1973 to 1976, less than 100 specimens per year were submitted for viral culture, and a virus was isolated from no more than 8 percent. Only herpes simplex virus (HSV), enteroviruses, and influenza viruses were isolated.

To improve our viral diagnostic capabilities, we decided to establish on-site laboratory services. The microbiology laboratory provides diagnostic procedures for all inpatients and a large, mostly lower socioeconomic, outpatient population at St. Francis Hospital and Medical Center (SFHMC), a 700-bed acute care community hospital offering all inpatient services except organ transplantation. The areas of microbiology (bacteriology, mycology, mycobacteriology, serology, and virology) are supervised by a doctoral-level clinical microbiologist and two supervisory-level medical technologists.

The virology section was established in 1976. Our approach was to utilize procedures that would detect the presence of a virus and in most cases provide a group identification. No attempt was made to develop full-service viral diagnostic facilities. Available tests include virus isolation in cell culture; direct viral antigen detection in clinical specimens for HSV, varicella zoster virus (VZV), respiratory syncytial virus, adenoviruses, and influenza A virus; isolate identification to group; and serologic tests for rubella, cytomegalovirus (CMV), VZV, and mumps virus antibody. The latter is used as an immunity screen by the SFHMC adolescent medicine clinic. Other serologic tests are performed at the Connecticut State Health Department (CSHD) Laboratory.

Initially a single technologist worked full time in virology. When chlamydia isolation was established (about 700 cultures per year) an additional half-time position was allotted to virology. Several other technologists have been trained to read cell cultures and immunofluorescent stains. All technologists in the laboratory can process specimens and recognize HSV-induced CPE in rabbit kidney (RK) cell culture. Thus genital cultures from obstetric patients are inoculated and read daily. Other specimens arriving on Saturday or Sunday are held at 4°C until Monday morning. Swab specimens are held in carrier medium (Hanks balanced salt solution at 4°C).

Commercially produced cell cultures (M.A. Bioproducts or Flow) used are human diploid fibroblasts (MRC-5), primary cynomolgous monkey kidney (CMK), Hep-2, and primary RK cells. McCoy cells for chlamydia isolation are passaged in the laboratory.

Isolates are identified to group by cell host range, CPE, and when possible by immunofluorescence. Positive cell cultures are delivered by a hospital driver to the CSHD laboratory for confirmation and serotyping. Physicians are notified of the presence of a virus by telephone as soon as antigen or CPE is detected.

Specimen transport is strictly controlled. Inpatient specimens must be delivered to the laboratory within 15 minutes of collection. Swab specimens are placed in cold carrier medium by laboratory personnel. Other specimens are held at 4°C until inoculated into cell culture. Outpatient specimens are accepted within 24 hours of collection if swabs are immersed in carrier medium and all specimens maintained at 4°C. The hospital clinics and a number of private physicians' offices are located in a separate building joined to the main hospital building by an over-the-street connector. A cold container with a rack of tubes containing viral and chlamydial carrier
media is delivered to the obstetrics and gynecology clinic daily. During the day clinic and private patient swab specimens are immersed in tubes of cold carrier medium and placed in the container. Laboratory personnel pick up the specimens in late afternoon, bring them back to the laboratory, and immediately inoculate them.

RESULTS

Since establishing the virology services, specimen volume has increased from the less than 100 per year sent to the reference laboratory to over 1,000 viral and 700 chlamydia cultures yearly. Between 12 and 24 percent of viral cultures and 15 percent of the chlamydia cultures are positive.

The distribution of specimens, the percentage positive, and the viruses isolated vary each year (Tables 1 and 2). For example, 7 to 12 percent of respiratory specimens were positive in 1980–1982, compared to 27 percent in 1983 (Table 1). Between 1980 and 1981, the number of genital specimens tripled. The percentage of isolates that were HSV also increased from 41 percent to a high, in 1982, of 90 percent (Table 2). The majority of genital HSV isolates are from female patients, many of whom are pregnant (Table 3). Viral isolates other than HSV are almost all from inpatients.

TABLE 2
Virus Isolates at SFHMC 1977–1983

| Virus isolated            | 1977–1980 | 1981   | 1982  | 1983 |
|---------------------------|----------|--------|-------|------|
| Adenovirus                | 12.1     | 5.4    | 0     | 4.7  |
| Influenza virus           | 9        | 2.2    | 0     | 4.3  |
| Parainfluenza virus       | 4.7      | 0      | 0     | 1.6  |
| Respiratory syncytial virus | 2.0    | 2.2    | .08   | 1.2  |
| Enteroviruses             | 29.7     | 11.8   | 6.7   | 7.4  |
| Herpes simplex virus      | 41.1     | 75.3   | 89.9  | 74.2 |
| Varicella zoster virus    | 2.3      | 3.2    | 1.7   | 3.5  |
| Cytomegalovirus           | 2.3      | 1.1    | 0     | 2.7  |
### TABLE 3
Sources of Herpes Simplex Virus Isolates—SFHMC 1977–1983

| Year Isolated | Oral/Respiratory | Lesion/Tissue/Other* | Female Genital | Male Genital | Total Isolates |
|---------------|------------------|----------------------|----------------|--------------|----------------|
|               | Inpatients | Outpatients | Inpatients | Outpatients | Inpatients | Outpatients | Inpatients | Outpatients | Inpatients | Outpatients | Inpatients | Outpatients |
| 1977–80       | 22        | 9          | 25        | 12          | 1       | 23           | 1       | 9          | 49        | 53          |
| 1981          | 14        | 2          | 10        | 7           | 6       | 30           | 0       | 1          | 30        | 40          |
| 1982          | 6         | 2          | 18        | 6           | 7       | 56           | 0       | 12         | 31        | 76          |
| 1983          | 26        | 1          | 15        | 28          | 2       | 99           | 1       | 18         | 44        | 146         |

*Eye, urine*
Inoculated cell cultures are read daily for an additional week. If CMV is suspected, cell cultures are held an additional two weeks. Hemadsorption on CMK cells with guinea pig red cells is performed twice the first week and on day 14 for detection of influenza and parainfluenza viruses. The majority (86 percent) of our isolates (other than HSV) are detected within the first week. Half are detected by 72 hours. Average days to first recognition of CPE or hemadsorption is 4.7. Herpes simplex virus produces CPE in our cell culture system in an average of 1.5 days (Table 4). By 48 hours, 84 percent of positive specimens are detected and, by 72 hours, 96 percent. A preliminary report is sent on all specimens submitted for HSV isolation after 72 hours' incubation.

**DISCUSSION.**

Data from other virology laboratories describing specimen and viral isolate distribution is available only through 1979. However, their patterns of specimen distribution and virus isolates are similar to our experience from 1976–1983. Assuming laboratory techniques used are appropriate, the mix of specimens submitted and viruses isolated still depends on a number of factors including variation in viral epidemiologic patterns, patient populations served by the laboratory, and physician interest.

Changes in specimen distribution have occurred in our laboratory as well as others. Between 1976–1977 approximately 38 percent of the specimens received in our laboratory, the Strong Memorial Hospital (SMH) laboratory [4], and the Mayo Clinic laboratory [5] were respiratory specimens. Since then our percentage of respiratory specimens has dropped to about 20 percent of the total. A similar drop (43 percent to 18 percent from 1968–74 to 1975–79) was seen at the West Haven Veterans Administration Hospital (WHVA) laboratory [6]. These changes were accompanied by a decrease in isolation of respiratory viruses. Two factors contributed to this change. Respiratory virus activity has been relatively low with no major influenza outbreaks. Also as physicians gain experience using the laboratory, fewer specimens are collected from outpatients with upper respiratory infection [5].

A change in enterovirus isolation was seen in our laboratory but not in the others. In 1977 a large outbreak of echovirus 6 infection occurred in Connecticut. That year, 60 percent of our total virus isolates were echovirus 6. No large outbreaks have occurred since, and enteroviruses account for 7 to 12 percent of all isolates—a figure
similar to that reported by Smith [5]. Although only 40 miles from SFHMC, the WHVA laboratory did not experience any increase in enterovirus isolation. This group of viruses represented only 1 to 2 percent of their isolates [6]. This difference is probably due to differences in patient population. Most clinical enteroviral disease occurs in children rather than in the adults seen at a VA hospital.

The increase in genital specimens and HSV isolation is nationwide and due to the increase in genital HSV infections and the wide publicity given this infection.

The percentage of positive specimens is also about the same from laboratory to laboratory. In general, the Mayo Clinic reported 13 to 14 percent positive [5], SMH 17 to 21 percent positive [4], the WHVA 13 percent positive [6], and SFHMC 12 to 24 percent positive. The increase at SFHMC is due to the increase in HSV isolation. In 1983, 13 percent of specimens other than genital or lesions were positive.

Although time to a report of viral detection is a major complaint of physicians, many viruses are detected in cell culture within one to four days [7].

According to McIntosh this is enough time to save patients days of both hospitalization and antibiotic therapy [7]. The average time to detection in cell culture in our laboratory for viruses other than HSV is 4.7 days. If direct antigen detection tests by immunofluorescence or enzyme immunoassay (Rotavirus) are included, average detection time of a positive specimen is 3.8 days, with 35 percent detected within 24 hours of collection.

At the SMH laboratory, enteroviruses are detected within 3.4 to 4.3 days [8] which is comparable to the 3.7 days for enteroviral isolation in our laboratory. However, the SMH laboratory now also inoculates BGM cells, an African green monkey kidney cell line that reduces recognition of coxsackie virus CPE by 1.4 to 1.6 days [9].

Time to detection of HSV in our laboratory is almost identical to that reported by Callihan and Menegus [10]. We both inoculate one tube of MRC-5 and one tube of RK cell cultures.

Cytomegalovirus had the longest detection time (9.3 days). This is longer by three days than the time observed by Gregory and Menegus [11]. In both laboratories 50 percent of positive specimens were found within one week. We both inoculate the same cell culture using similar techniques. Thus, longer detection time for CMV in our laboratory is probably because more of our virus-positive specimens were from adults than from infants. Infants usually shed large amounts of virus and CPE generally develops rapidly—often within a few days.

Laboratory utilization by clinicians has grown and improved. Rarely are specimens for culture or immunofluorescence submitted from patients with a clear-cut clinical diagnosis such as typical VZV or oral herpes infection. A large percentage of the genital specimens submitted are to diagnose and monitor genital HSV infections during pregnancy. In the last year three cases of HSV encephalitis were diagnosed by direct immunofluorescence on brain tissue within two hours of biopsy. On histologic examination only two of the brain biopsy specimens contained viral inclusions, but HSV was isolated from all three within 48 hours.

Requests for pre- and postnatal serologic screens for Toxoplasma gondii or CMV antibody have decreased. We have discouraged physicians from ordering these as screening tests because of their questionable clinical value [12]. Neonatologists now order appropriate cultures for viral isolation or consult with the laboratory before attempting to make a serologic diagnosis.

Finally, control of nosocomial viral infections has been enhanced. For example,
determination of employees' immune status to VZV allows epidemiologists to excuse only non-immune employees from work following VSV exposure in the hospital. By fully using our reference laboratory (CSHD) to serotype viral isolates, to do most diagnostic serologic tests and some special procedures, we are able to keep our costs down while providing optimum virologic diagnostic services.

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