The Functions of a University-Based Viral Diagnostic Laboratory: Recent Experiences at Yale–New Haven Hospital

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The work of the Viral Diagnostic Laboratory at Yale–New Haven Hospital has been continuously inspired by the virologic and epidemiologic principles taught us by Dr. Horstmann. This paper helps to define the various functions of this facility. Perhaps its most important role is as a learning laboratory where generations of students, fellows, house officers, and teachers are taught how viruses behave in the human host and in the community.

THE FUNCTIONS OF A UNIVERSITY-BASED VIRAL DIAGNOSTIC LABORATORY

The viral diagnostic laboratory at the Yale–New Haven Hospital serves three major functions: service to the hospital and to the community, teaching, and research. These functions are inextricably intertwined. Each specimen provides us with a challenge in diagnosis which will ultimately have an impact on the management of a patient, affords us a valuable opportunity to teach students and house officers about the epidemiologic and clinical behavior of viruses, and sometimes offers us the chance to think about the pathogenesis of viral disease in a novel way and to suggest new avenues of clinical research. In order to accomplish these tripartite goals most effectively, the viral diagnostic laboratory needs a supervisor, preferably a clinician, who is well-versed in the clinical aspects of a wide assortment of viral diseases and who is also familiar with viral diagnostic methodologies and basic epidemiologic principles.

The discipline of viral diagnosis is time-consuming and relatively expensive and the laboratory cannot accept uncritically all specimens which may come its way. In this aspect, it is different from most other hospital laboratories which routinely process myriad specimens without regard to their source and without asking whether the test is appropriate to the clinical questions being asked. We strongly encourage the clinicians to consult with us by telephone or in person before any specimens are collected. After discussing the case with the physician, we can offer cogent advice about which specific specimens are most appropriate, and we can give instructions about the collection of the material and its inoculation into tissue culture. We encourage the doctors to obtain viral cultures as early in the clinical illness as possible and to...
make arrangements to have them brought to the laboratory as quickly as possible. In some instances we reserve the right not to undertake work on a given patient or specimen if the yield is felt to be too low or if the expected results cannot be adequately interpreted. When consultations take place, the referring physician learns something about the epidemiology and behavior of viruses, and various techniques used in diagnosis. In addition, the clinician will often learn to focus his thinking on the several etiologic agents which are most likely to be playing a role in the patient's illness rather than on the numerous agents that initially sprang to mind. For many students and house officers, detailed communication with the diagnostic laboratory in the context of specific patients with specific viral illnesses provides the most dramatic and relevant teaching about viruses which they are likely to encounter in their years of training.

The laboratory provides concrete information which has both prognostic and therapeutic importance. For example, by informing the physician that his patient has meningitis of viral rather than bacterial etiology, we help the clinician to prognosticate and to alter his treatment plan—expectant antibiotic therapy can be terminated, and the patient can be sent home to recuperate. When we isolate Herpes simplex from the cervix of a woman in her thirty-eighth week of gestation, we can comfortably advise the obstetrician to consider delivery by Caesarian section in order to prevent disseminated infection in the newborn. Finally, in discovering the actual cause of an illness, the clinician is treated to a rare, but very invigorating experience.

Daily Activity in the Diagnostic Virology Lab: Specimens Received and Isolations Made

The viral diagnostic laboratory of the Yale-New Haven Hospital operates on a modest scale. All of the day-to-day work is done by one highly experienced research associate, Mrs. Grace Tucker, in a space of 180 square feet which is located in a much larger virus research facility. She prepares virtually all of her tissue cultures, receives and logs in all specimens, inoculates culture tubes and reads them for cytopathic effect, identifies and types isolates, participates in some small research projects, contacts the referring physician or the clinical fellow when a tentative isolate has been made, keeps detailed records, and sends out all final reports. As supervisor of the laboratory, I give advice about the handling and work-up of particular specimens, and act as the primary intermediary between the clinicians and the lab, especially in helping to interpret positive results.

The medical center has over 800 beds. In the past five years the number of specimens we receive has almost doubled (refer to Table 1); we presently process materials from more than 100 patients per month. Nearly 16 percent of all specimens received yield a virus isolate, and we can ascribe a viral etiology to the illnesses of nearly one in every four to five patients from whom we receive clinical material. This favorable yield is in part due to tighter control over distribution of our culture tubes and a more intimate relationship between the house staff and the laboratory. Especially where pediatric patients are concerned, we generally know something about each of them and the diagnoses that are being considered. In addition, our increasing commitment to educational programs and lectures in the hospital and in the medical community at large has cultivated a more sophisticated and well-informed group of physicians, at least as far as viruses are concerned.

Approximately 20–25 percent of our yearly volume comes from the pediatric service. Although we are most intimately involved with the pediatricians on a daily
basis and although the greatest *variety* of isolates comes from hospitalized infants and children, the greatest *number* of specimens comes from adults. The reason for this will become apparent from the data shown in Table 2. The most common anatomic sites cultured are the skin and genitalia. In the past two years, 40-50 percent of the laboratory's work has been primarily concerned with the etiology of lesions from these sites, and the overriding concern is usually Herpes simplex. Of late, a greater proportion of such specimens come from obstetrician-gynecologists in private practice. This trend quite clearly mirrors the recent statistics published by the Centers for Disease Control which shows a striking increase in the number of office visits to private physicians for genital herpes during the 1970s [1]. Concern over genital herpes is also fired in the community of patients and physicians by the extraordinary media blitz of recent months, consisting of a cluster of reports concerning this affliction by the New York *Times* Magazine, 60 Minutes, and the MacNeil-Lehrer Report.

The other fifty percent of our material comes mainly from the pediatric and adult medicine services where the major concern is diagnosis of various central nervous system ailments, especially encephalitis and aseptic meningitis; acute febrile illnesses of unclear etiology; lower respiratory tract disease, including bronchiolitis, bronchopneumonia, and interstitial pneumonia; mononucleosis syndromes; various exanthema; and congenital or neonatal infections.

### TABLE 1
Number of Specimens Received and Number of Patients in Whom a Viral Diagnosis Was Sought, 1975-1981

| Year | No. Specimens | No. Patients | No. Isolates | % Specimens Positive | % Patients with Viral Diagnosis |
|------|---------------|--------------|--------------|----------------------|--------------------------------|
| 1975 | 1106          | 819          | 102          | 9.2                  | 12.5                           |
| 1976 | 812           | 634          | 91           | 11.2                 | 14.5                           |
| 1977 | 791           | 604          | 130          | 16.4                 | 21.5                           |
| 1978 | 866           | 632          | 143          | 16.5                 | 22.6                           |
| 1979 | 1246          | 910          | 238          | 19.1                 | 26.2                           |
| 1980 | 1244          | 969          | 183          | 14.7                 | 18.9                           |
| 1981 | 1522          | 1233         | 242          | 15.9                 | 19.6                           |

### TABLE 2
Clinical Specimens Received by the Viral Diagnostic Laboratory, Yale-New Haven Hospital, *By Site Cultured*

| Site      | Nasopharynx | Throat | Rectum | CSF | Eye | Skin and Genitalia | Miscellaneous |
|-----------|-------------|--------|--------|-----|-----|--------------------|---------------|
| 1975      | 99 (9.0)    | 154(13.9) | 173(15.6) | 155(14.0) | 119(10.8) | 257(23.2) | 149(13.5) |
| 1976      | 47 (5.8)    | 146(18.0) | 115(14.2) | 70( 8.6)  | 82(10.1) | 190(23.4) | 195(24.0) |
| 1977      | 34 ( 4.3)   | 132(16.7) | 88(11.1)  | 43( 5.4)  | 90(11.4) | 283(35.8) | 119(15.0) |
| 1978      | 77 ( 8.9)   | 130(15.0) | 112(12.9) | 39( 4.5)  | 87(10.0) | 272(31.4) | 149(17.2) |
| 1979      | 194(15.6)   | 153(12.3) | 189(15.2) | 110( 8.8) | 50( 4.0) | 408(32.7) | 134(10.8) |
| 1980      | 142(11.4)   | 127(10.2) | 166(13.3) | 82( 6.8)  | 32( 2.6) | 549(44.1) | 143(11.5) |
| 1981      | 124( 8.1)   | 99( 6.5)  | 151( 9.9) | 100( 6.6) | 12(< 1)  | 782(51.4) | 234(15.4) |

( ): Numbers in parentheses refer to percentage of total specimens received from that site. Miscellaneous includes: urine, white blood cells, biopsy and autopsy specimens, bronchial brushings, inflammatory fluids.
It is not surprising, based on the most common sites cultured, that our most frequent isolate is Herpes simplex virus (Table 3). In fact, of late, 70–80 percent of all our isolates are positive for Herpes simplex virus. The various enteroviruses constitute the next greatest number of isolates; almost all of these come from pediatric patients who present with disease of the central nervous system.

Our more recent increased success in isolating RSV and cytomegalovirus (CMV) can be attributed to better collection of specimens and more rapid transport to the lab. We have encouraged the pediatric house staff to collect specimens for isolation of respiratory pathogens by aspirating the contents of the posterior nasopharynx using a narrow gauge gavage tube attached to a 5 ml syringe [2] and to inoculate such specimens immediately, at the bedside, using a fresh tissue culture tube. The house staff also collects multiple urine specimens from patients in whom they suspect infection with CMV. Since CMV is excreted sporadically, this method, along with prompt hand delivery of specimens to the lab, probably accounts for our increasing number of isolates of this agent.

Although we do a reasonable job of isolating respiratory syncytial virus, we are less successful with other respiratory viruses. The dearth of myxovirus isolates probably reflects the fact that most patients from whom we receive specimens are in the hospital for complicated and progressive disease, at a time when viral shedding has long since ceased. As Dr. Horstmann and Dr. Denny and their associates have shown, surveillance for respiratory pathogens is best done in a private pediatric practice or in the outpatient department of a hospital [3,4]. Continuous viral surveillance in such settings quite admirably reveals the prevalent types of viral infections in the community at any given time. As Dr. Horstmann has stated, “young children with acute illness who come from various parts of the city or area in a sense can be considered as 'sentinels' indicating what agents are on the scene, and how extensively they are circulating” [3]. In the case of respiratory agents, especially influenza, such children often alert the public health authorities to the emergence and subsequent spread of new antigenic variants.

Special Tests and Studies

The viral diagnostic facility occupies space in a large research laboratory that is primarily concerned with the biology of human herpesviruses, especially Epstein-Barr virus. Because of our interest in this agent, we have incorporated EB virus serologic and virologic diagnostic procedures into the workings of the laboratory. Although we have been primarily interested in the unusual and atypical ways in which primary EB virus infections present in childhood, we also investigate mononucleosis-like illnesses in adolescents and adults who do not develop a heterophile antibody response, or who have prolonged or recurrent symptoms, or some major complication of their illness, such as hepatitis or encephalitis.

Recently, we have been doing more work with varicella-zoster virus (VZV). This was initially prompted by the concern of the pediatric hematologists and oncologists over the life-threatening infections produced by VZV in their severely compromised patients. We now routinely screen the bloods of all our pediatric oncology patients, as well as pediatric nurses at the time they are hired, for antibody to the VZV-induced membrane antigen (FAMA test). Knowing the susceptibility to chickenpox of these individuals, we can act promptly and decisively when any of them is exposed to an acute case. We also use the results of the FAMA test to select patients with acute lymphocytic leukemia in remission as potential recipients of a new live attenuated varicella vaccine that is presently undergoing trials in the United States.
### TABLE 3
Specific Viral Isolates Made, by Year and Class, 1975-1981

| Year | HSV | VZV | CMV | Echo | Cox | Polio | Not Sero-typed | Adeno | Flu A | Flu B | RSV | Parafu | Rhino | Rotavirus† |
|------|-----|-----|-----|------|-----|-------|---------------|-------|-------|-------|-----|--------|-------|------------|
| 1975 | 59(58)* | 7 | 2 | 12 | 6 | 6 | (24)* | 4 | 3 | — | — | 3 | — | — |
| 1976 | 63(69) | 6 | — | 8 | 3 | 1 | (13) | 4 | 3 | — | 1 | 2 | — | — |
| 1977 | 90(69) | 15 | 7 | 6 | 1 | 5 | (5) | 3 | — | 2 | 1 | 3 | 2 | — |
| 1978 | 97(68) | 10 | — | 7 | 4 | 3 | (10) | 8 | 1 | — | 9 | — | 4 | — |
| 1979 | 150(63) | 16 | — | 45 | 11 | 4 | (25) | 4 | 1 | — | 6 | 1 | — | — |
| 1980 | 150(82) | 12 | 2 | 7 | 6 | 1 | (8) | — | — | — | 3 | — | 1 | — |
| 1981 | 199(82) | 12 | 15 | 12 | 4 | 10 | 3 | (12) | 1 | — | — | 8 | 1 | — | 5 |

*Numbers in parentheses represent percentage of all isolates that were positive for Herpes simplex or for all types of enteroviruses.
†Rotavirus antigen is detected in an ELISA test.
THE VIRAL DIAGNOSTIC LABORATORY AS A TEACHING FACILITY IN INFECTIOUS DISEASE PATHOGENESIS AND EPIDEMIOLOGY

We are now well-accustomed to and constantly delighted with the variety of ways in which the agents isolated from specific patients give us new ideas and insights about the ways in which viruses behave in the individual host, as well as in the community. Occasionally, specific isolates verify the observations made by other clinician-investigators in the medical literature and serve as exciting clinical paradigms for students, house officers, and attendings.

I would like to end this commentary by providing examples of a number of just such exhilarating virologic paradigms which have been provided us in the past few years. The lessons we have learned from these patients follow each clinical vignette.

**Case 1.** A five-month-old black infant was admitted to the Hospital of Saint Raphael with cough, fever, and difficulty breathing. There were retractions, profuse, thick nasal secretions, and otitis media. The white blood cell count was elevated and there was a lymphocytosis with 30 percent atypical forms. A Monosticon “spot test” for diagnosis of infectious mononucleosis was weakly positive. In order to confirm the diagnosis of acute IM presenting in the form of lower respiratory tract disease, peripheral blood lymphocytes were co-cultivated with human umbilical cord leukocytes on fibroblast feeder layers in a standard transformation assay. After four weeks, there was no evidence of transformation, but cytopathic effect (CPE) was observed in the fibroblasts. The agent could be passed and showed typical adenovirus CPE in HEP-2 cells. The virus was identified as adenovirus type 2. The same agent was recovered from a tracheal aspirate obtained three weeks after the leukoviremia was found, and the patient developed neutralizing antibodies in the course of the illness [5].

**Lessons:**

*a.* Sometimes, in the course of looking for one thing, we discover something quite unexpected.

*b.* Adenoviruses can be recovered from the mononuclear fraction of heparinized blood from whence they may be induced to replicate by conditions of co-cultivation.

*c.* Transport of specific adenoviruses in circulating white cells could account for their dispersal to and persistence in lymphoid tissue throughout the body.

**Case 2.** A 17-month-old infant was studied who presented with upper respiratory infection, cervical lymphadenopathy, injected tympanic membranes, palatal petechiae, tonsillar hypertrophy, thrombocytopenia, and atypical lymphocytosis. Platelet count was 4,000/mm³. A Monosticon “spot” test was negative and Paul-Bunell heterophile antibodies were absent. He was treated with ampicillin and prednisone. One month following discharge the platelet count was 300,000/mm³. The patient had serologic evidence of primary infection with EB virus and transforming virus was isolated from throat on the twentieth and thirty-third days following onset of illness [6].

**Lessons:**

*a.* Infants and young children experiencing primary EBV infection rarely present with classical symptoms of infectious mononucleosis and only occasionally develop heterophile antibodies. The diagnosis of primary EBV infection was initially entertained in this patient only when 30 atypical lymphocytes were seen on the blood smear.

*b.* Thrombocytopenia and other hematologic disorders, such as hemolytic
anemia, are among the clinically apparent manifestations of EB virus infection in the young child. The manner in which EBV induces these hematologic abnormalities requires further investigation.

Case 3. A 77-year-old woman presented to Yale-New Haven Hospital with zoster, fever, lethargy, and confusion. The neurologic signs expressed themselves approximately two weeks after the onset of thoracic zoster. On admission the patient had fever, vesicles, and superficial sloughing over the left fourth and fifth thoracic dermatomes, loss of short-term memory, inability to interpret proverbs, and decreased strength in both lower extremities. Lumbar puncture revealed a mild mononuclear pleocytosis; this resolved over the next four days. Mild memory deficit and fatigue were her only residual symptoms at discharge. Varicella-zoster virus was recovered from unspun CSF on both the fourth and seventh days after the onset on neurologic disease. Antibodies to VZV-induced membrane antigen (FAMA) were present in CSF in a titer of 1:64; serum antibodies were at least 16-fold higher. Evidence for local antibody production was derived from calculation of a CSF-IgG index [7].

Lessons: 

a. Zoster encephalitis may occur in otherwise healthy individuals, without underlying malignancy or immunodeficiency.

b. It may take as long as two weeks before VZ virus reaches the CNS and replicates sufficiently to evoke signs of neurologic dysfunction.

c. The presence of VZV-specific antibodies in CSF probably reflects local production, as suggested by Gershon and colleagues [8]; further proof of local antibody production can be obtained by comparing the concentration of IgG and albumin in serum and CSF obtained at the same time.

Case 4. A one-month-old baby boy who was the twin A product of a 36-week uncomplicated pregnancy developed rhinorrhea and cough. His 20-month-old sister had a similar illness. The patient's cough was harsh but not paroxysmal. He developed post-tussive spitting and pallor. On admission to Danbury Hospital the respiratory rate was noted to drop frequently from 90/minute to 60/minute. Chest X-ray was unremarkable. Periods of apnea, accompanied by pulse drops, were soon noted. Upon transfer to Yale, the baby required nearly continuous stimulation to control apnea. He also had retraction, fine crepitant rales, opisthotonic posturing, hyperreflexia, and jitteriness.

Concurrent with Twin A's transfer to Yale, Twin B became ill with cough and stuffy nose. Within 24 hours she developed apneic episodes lasting 10-15 seconds. On arrival at Yale she had frequent episodes of apnea, pulse drops, and arching posture. Both infants responded to frequent suctioning and O2 given by headbox. They were discharged together on Twin A's tenth hospital day.

Nasopharyngeal cultures on both twins and the father, who also became ill four days after Twin B's hospitalization, grew respiratory syncytial virus (RSV).

Lessons: 

a. RSV sometimes causes life-threatening infections in infants. Bronchiolitis is an unusual finding in infected neonates, but apnea is a common presenting sign [9].

b. RSV is most likely to be introduced into a family by an older child who is experiencing a re-infection; in this instance, the 20-month-old sister may have initiated this family-wide outbreak.
c. It is very helpful to trace the epidemiologic patterns of virus spread by culturing other members of the family. Recovery of an agent from a family member experiencing illness at a time when the patient is no longer shedding virus may give a clue to the etiology of the patient's illness.

Cases 5 and 6. Cytomegalovirus (CMV) was isolated from the urine of a five-week-old who was afebrile and who presented with a three-day history of rhinorrhea and cough. The chest X-ray showed diffuse patchy infiltrates. CMV was also recovered from multiple urine samples of a one-month-old with afebrile pneumonia whose symptoms included tachypnea, retractions, other signs of lower airway obstruction, and staccato, hacking cough. Both infants recovered uneventfully.

Lessons: a. CMV pneumonitis may occur in otherwise normal infants who are not immunosuppressed.

b. Neonatal pulmonary infections due to CMV are probably acquired from the maternal genital tract at the time of birth by aspiration of infected secretions. The long incubation period of infections due to CMV causes the pneumonia to become clinically apparent only after four to six weeks [10].

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