A USER'S GUIDE TO THE DIAGNOSTIC VIROLOGY LABORATORY

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Preface

While I was developing a clinical virology diagnostic service I was often surprised by the problems and uncertainties experienced by many physicians in submitting specimens to the laboratory. At the same time, I was aware that many other physicians were consistently able to submit specimens that frequently gave evidence for viral infections, although not always the infections suspected by the submitters. In considering how some people made effective use of the virology laboratory while others did not, I found that the clinicians who used the laboratory most regularly tended to obtain the best results and were also the best informed about laboratory services and details of specimen collection and submission. The well-informed clinicians made it their business to learn about laboratory services in advance of their need to use the various services. It became clear that cooperation and good communications between the clinician and the laboratorian are keys to the use of the virology laboratory as an aid in patient care. Physicians need to be informed about 1) the laboratory tests available to them, 2) which specimens are appropriate to submit for different conditions, 3) when and how to collect and transport these specimens, and 4) what information is needed by the laboratory staff to provide effective service. All of the information can then be used when the clinician keeps in mind definite reasons for requesting the various laboratory tests.

Unfortunately, much of this information never seems to be at hand for the occasional user of the laboratory. Detailed information on most of these points is found in laboratory reference manuals, rather than in the clinician's pocket. Although laboratorians recognize an obligation to provide useful information to the users of the laboratory, the instructions and write-ups that have been developed are often too bulky or inconvenient to carry around—thus the need for a pocket-sized guide to virology services.

Although there is adequate consensus among clinical virologists about specimen collection and submission to make a guide practical, it should be recognized that different laboratories follow somewhat different procedures, and that a printed guide will not substitute for consultation with the supervisor or director of the laboratory, especially when unusual circumstances arise. If one allows for such limitations, this guide may prove useful in improving the utilization of clinical virology laboratories. As this publication is only a modest beginning, there will certainly be room for improvement, and I will be grateful for any corrections and suggestions that the readers can offer.

D.A.L.

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Contents

Introduction

REASONS FOR OBTAINING LABORATORY DIAGNOSIS

The objective of this guide is to provide the clinician with assistance in obtaining effective and specific laboratory diagnosis of viral, rickettsial, chlamydial, and mycoplasmal infections. The latter three groups of organisms are included because tests for detecting infections with these agents are usually carried out in a "virus" laboratory. Before one uses any laboratory services, however, it is important to have in mind the reasons for acquiring information by means of laboratory testing. Some of the reasons for obtaining laboratory diagnosis of viral infections are:

1. To confirm and improve clinical diagnosis and judgment
2. To determine an etiology for a currently prevalent syndrome, for epidemiologic and patient-education purposes
3. To obtain a more accurate prognosis
4. To permit the application of appropriate prophylactic measures, if nosocomial or community spread of an infection is threatened
5. To provide a basis for implementing appropriate antiviral therapy, and/or ceasing inappropriate therapies

Improving Clinical Diagnosis

While it is true that a number of the more common viral diseases can be diagnosed from clinical observations alone, it is also true that many viral diseases may be difficult to diagnose without the aid of a laboratory. Viral diseases may be clinically similar to conditions resulting from bacterial or mycotic infection, or even conditions of non-infectious etiology. Common viral illnesses may present sufficiently unusual symptoms to prevent their prompt recognition, e.g., the "atypical" measles syndromes produced in individuals who have developed incomplete immunity to measles following vaccination. In contrast, some well-recognized viral diseases may be encountered so infrequently as to make laboratory documentation of their etiology seem advisable, e.g., the hemorrhagic cystitis syndrome, which may be caused by several serotypes of adenoviruses.

Providing Epidemiologic and Patient-education Information

In general, physicians desire to be able to give their patients specific and accurate diagnoses. When diagnosis includes the name of the virus that is "responsible," it will contribute to an increased medical literacy of the public, and may increase patients' confidence in their physician. By selective virologic testing of only a few patients, a physician in general practice may be able to offer patients the assurance that their illnesses are most likely due to ECHO virus, type 9, rather than the vague attribution to "some virus that is currently going around." The latter comment is unlikely to inspire any confidence that the physician is more knowledgeable than the patient.

Accurate Prognosis

The prognoses of clinically similar conditions with different etiologies can be radically different. Meningoencephalitis produced by mumps virus infection is benign, while that due to herpes simplex virus infection is not. Some cases of infectious mononucleosis (due to the Epstein-Barr virus) remarkably resemble some forms of leukemia. Without laboratory information, assessment of the patients' conditions cannot be made. We are still learning about the long-term prognosis for some viral infections, as laboratory-supported studies reveal sequelae of infections previously regarded as unimportant. Without specific, laboratory-confirmed diagnosis of viral infections, such studies could not be carried out.

Prophylaxis and Therapeutic Considerations

Accurate diagnosis of communicable diseases often allows implementation of appropriate measures to prevent further spread of infection. Prophylactic measures may be initiated on behalf of individuals at high risk of developing serious illness, or to prevent nosocomial illness within an institution, or to stem epidemic disease within a community. A leukemic child exposed to a person with varicella and a pregnant woman exposed to a person with rubella are individuals at high risk of serious consequences from otherwise minor illnesses. The leukemic child can be protected against developing severe, possibly fatal

varicella by the administration of zoster immune globulin within four days after exposure; the pregnant woman, if found non-immune to rubella, may choose to terminate her pregnancy if she develops evidence of infection with rubella. The discovery of a communicable disease, such as varicella, in a hospital will frequently result in action by the hospital's infection control committee to prevent an outbreak from developing. The measures taken may include various forms of quarantine, restricted admissions (admitting only patients with demonstrated immunity to the threatening infection), and possible discharge or passive immunization of certain susceptible or immunocompromised patients. Under these conditions, the serologic assay of immune status of those exposed to the source of infection is useful in deciding upon a course of action.

AVAILABILITY OF DIAGNOSTIC VIROLOGY SERVICES

The extent and accessibility of viral laboratory services vary considerably in different geographic areas, and change frequently. In the past, most state public health laboratories were the principal providers of viral diagnostic services when such services were largely unavailable from private laboratories. In some states, the public health laboratory is still the main source of diagnostic virology services. In other states, public health laboratories continue to provide these services only to obtain important epidemiologic information for the control of communicable diseases. Recently, there has been a trend to reduce the level of clinical virology services offered by various state health departments, coordinated with efforts to increase the availability of services at the local laboratories. Local or regional viral diagnostic services may be provided by city or county public health laboratories, by larger hospital laboratories, or by commercial laboratories. The privately operated laboratories are often better prepared to provide clinically useful diagnostic services, as the usual role of public health laboratories is to provide reference testing, training, proficiency testing programs, and support for epidemiology and communicable disease control programs. Some larger state (and private) laboratories undertake to develop and to evaluate new laboratory technologies. The

number of privately operated diagnostic virology laboratories has been increasing substantially in recent years. Appendix A provides a list of laboratories in the United States that offer diagnostic virology services, and indicates which laboratories are licensed to accept interstate shipment of specimens.

LABORATORY CONSULTATION

One of the services that many laboratories can provide, in addition to performing diagnostic procedures, is consultation for clinicians to determine the most appropriate tests and specimens to meet particular diagnostic needs. The less familiar that a clinician is with a particular laboratory specialty, the more likely it is that such consultation is useful and important. However, laboratory methods change, and new tests are introduced from time to time, so even experienced clinicians may find consultations with laboratory staff to be useful.

Clinicians should consider consultation with the laboratory director to be advisable when:

1. Results from the laboratory are repeatedly unsatisfactory for any reason.
2. The laboratory frequently requests either more clinical information or additional specimens, or both.
3. It is unclear whether routine procedures can provide the information desired.
4. It is not known whether any specific viral infections are associated with an unusual syndrome.

For a virology laboratory to perform the most appropriate tests, and to be able to interpret the results of these tests to the interested clinician, a certain amount of clinical information is usually required by the laboratory staff. Fortunately, most of the information that is required routinely will be supplied if the clinician will fill out a few lines on the laboratory specimen submission form. The information requested will usually make consultation unnecessary, but may alert the laboratory staff to the need for further follow-up with the clinician.

PART I

Laboratory Methods for Diagnosis of Viral Infections

There are three approaches available for the laboratory diagnosis of viral infections: the direct detection of viruses from clinical specimens, the isolation of viruses from the same specimens, and serologic tests of paired sera collected during the acute and convalescent phases of an illness. In addition, serologic tests of single serum specimens may provide information about immunity to specific viruses of interest.

DIRECT DETECTION

Direct Immunofluorescence

Direct immunofluorescence methods are used to detect viral antigens present in cells obtained *directly* from an appropriate specimen.

Advantages These tests are rapidly completed, so that results may be available only a few hours after the specimen is processed in the laboratory. Viruses are identified at the same time that they are detected, and antigens may be detectable even when infectious virus is no longer present.

Test Requirements The specimen submitted must contain cells suitable for staining, and the submitter must specify the viruses that are likely causes of the illness. Only a few different viruses may economically be tested for any one specimen, so the number of tests requested must be limited. It is not feasible to test for the presence of some viral groups consisting of many serotypes (e.g., the enteroviruses), or those for which no reagents are available. The likelihood

of obtaining positive results is greatest when the specimens are collected early during the course of an illness.

Immunoperoxidase Methods

Immunoperoxidase methods and other immunologic staining methods can be used in the same manner as immunofluorescence testing.

Electron Microscopy

Electron microscopy methods are used most often to detect "free" virus particles (particles not inside cells) obtained from an appropriate specimen, but they can also be used to detect intracellular viruses. The sensitivity of most electron microscopy methods is rather low, meaning that the concentration of virus particles must be quite high before they can be reliably detected. However, immunoelectron microscopy, which uses specific antiviral serum to concentrate virus particles into clumps, is somewhat more sensitive.

Advantages The simple methods used for virology specimens allow tests to be completed rapidly, so that results may be available an hour or so after the specimen is received in the laboratory (unless the specimen must be concentrated). A number of different viruses that cannot be grown in cell cultures, or that can be grown only with difficulty, can be detected and identified by electron microscopy. As viruses with different morphologies may be readily distinguished from each other, the submitter need not specify the particular viruses to be sought. However, viruses with the *same* morphology cannot usually be distinguished from each other, e.g., HSV and VZV.

Test Requirements The specimens that can be tested are limited to those that either contain virus particles at high concentration (e.g., vesicle fluid), or that can readily be concentrated to yield a high concentration of virus particles (e.g., urine). Access to an electron microscope may be limited, so priority will usually be given to specimens that are suspected to contain viruses that are difficult or impossible to cultivate.

Significance of Direct Detection Test Results

Positive results obtained by direct detection methods are usually judged to be very significant when they are correlated with disease occurring adjacent to the specimen site. When disease occurs in parts of the body remote from the specimen site, the significance of positive results is much less certain, and the results must be considered in view of other clinical information.

Negative results are often not considered to be significant for several reasons:

1. The test used may not be sensitive enough to detect small amounts of virus or viral antigens.
2. Intermittent shedding of virus (found with latent herpes simplex infections, for example) may not be detected.
3. Immunologic tests may not have included the correct agent among those tested for.

ISOLATION

Virus Isolation

Virus isolation is the recovery of infectious virus(es) from appropriate specimens collected early in the course of an illness. Several types of living hosts may be used in attempts to isolate different kinds of viruses. The most commonly used are cell cultures, but embryonated eggs and infant mice are used for certain viruses.

Advantages A wide range of viruses may be detected in suitable cell cultures without the need to specify which viruses are to be sought. Certain viruses can be isolated and identified for which direct tests and serologic tests are not available, e.g., the enteroviruses. Viruses may be isolated that are present in numbers too small to be detected by direct methods.

Test Requirements Because some viruses require the use of hosts that may not be in routine use by the laboratory, it is advisable to tell

the laboratory which viruses are suspected to be causing an illness. As with the direct detection methods, the likelihood of isolating a virus is greatest when specimens are collected early during an illness. The physician should be aware that, as yet, some viruses cannot be grown in the laboratory. Although many viruses are detected within 3–5 days, an isolation attempt is usually continued for about two weeks before being discarded as negative. Therefore, the isolation of viruses is often regarded as a somewhat "slower" procedure than the cultivation of most bacteria.

Special Isolation Methods

Special isolation methods are available for *Chlamydiae* and, less commonly, the *Rickettsiae*—bacteria-like organisms that, like viruses, are obligate intracellular parasites. Chlamydial isolation is becoming available in many virology laboratories, as the cell culture facilities needed for these organisms are usually available in these laboratories. Rickettsial isolation, which is performed in such hosts as guinea pigs or embryonated eggs, requires special facilities and is usually available by arrangement with a larger public health laboratory.

Chlamydial Isolation Chlamydial isolation is required for the diagnosis of most infections with *C. trachomatis,* as the serologic test used for diagnosis of lymphogranuloma venereum or ornithosis is not sensitive enough for these other infections. The isolation procedure usually requires 2 or 3 days. Isolation of *Chlamydiae* is unlikely once patients are placed on effective antibiotic therapy.

Rickettsial Isolation Rickettsial isolation is seldom required for the diagnosis of rickettsial infections, which are effectively diagnosed by serologic tests. The isolation of *Rickettsiae* involves substantial risk of infection to laboratory workers, and in most laboratories it is not undertaken unless special containment facilities are available. The procedures used for rickettsial isolation recently have been instrumental in the recovery and identification of several newly recognized bacteria that cause respiratory disease. It is likely that these procedures will continue to be used for research on the etiology of such illnesses, but not for their routine diagnosis.