Chapter 19: Laboratory Support for the Surveillance of Vaccine-preventable Diseases

Hayley Hughes, MPH; Melinda Wharton, MD, MPH

I. Surveillance of vaccine-preventable diseases

Surveillance for vaccine-preventable diseases requires the close collaboration of clinicians, public health professionals, and laboratorians. Public health surveillance relies on clinical and laboratory reports of vaccine-preventable diseases (VPDs). Therefore, appropriate specimen collection and laboratory testing is essential. This chapter provides guidelines on which specimens to collect for each vaccine-preventable disease and how to interpret laboratory results.

Each public health professional dealing with vaccine-preventable diseases should identify sources of laboratory support for his or her clinical and public health practice. **Table 1** lists appropriate tests for VPDs and provides a worksheet for recording laboratories and laboratory personnel. In addition to the guidelines presented in this chapter, state health department personnel can provide additional guidance on specimen collection, transport, and other information.

II. General guidelines for specimen collection and laboratory testing

Specimen collection and shipping are important steps in obtaining laboratory diagnosis or confirmation for VIPs. Many publications provide guidelines for specimen collection, storing, shipping and laboratory testing for viral and microbiologic agents.[1,2] The CDC has compiled information on using the CDC laboratories as support for reference and disease surveillance (RDS).[3] This publication contains the form required for submitting specimens to the CDC (CDC 50.34) (**Appendix 24**) and information on general requirements for shipment of etiologic agents (**Appendix 25**). Although written to guide specimen submission to CDC, this publication as well as other guidelines for collecting, processing, storing, and shipping diagnostic specimens (**Appendix 26**) may be applicable to the submission of specimens to other laboratories.

III. Disease-specific guidelines for specimen collection and laboratory testing

This chapter provides a quick reference of the laboratory information from Chapters 1–14 of this manual. **Table 2** lists confirmatory and other useful tests for the surveillance of vaccine-preventable diseases, and **Table 3** summarizes specimen collection procedures for laboratory testing. Because some specimens require different handling procedures, be sure to check with the diagnostic laboratory prior to shipping. When in doubt about what to collect, when to collect, where to send specimens, or if you have other related questions, call the state health department and laboratory.

A. Diphtheria (see Chapter 1)

Diagnostic tests used to confirm infection **include isolation of *C. diphtheria* on culture** and **toxigenicity testing**. Although no other tests for diagnosing diphtheria are commercially available, CDC can perform a **polymerase chain reaction** (PCR) test on clinical specimens to confirm infection with a toxigenic strain. The PCR test can detect non-viable *C. diphtheriae* organisms from specimens taken after antibiotic therapy has been initiated.

Although PCR for the diphtheria toxin gene and its regulatory element, as performed by the CDC Diphtheria Laboratory, provides supportive evidence for the diagnosis, data are not yet sufficient for PCR to be accepted as a criterion for laboratory confirmation. At present, a case that is PCR positive without the isolation of the organism or histopathologic diagnosis and without epidemiologic linkage to a laboratory-confirmed case should be classified as a probable case.

Isolation of C. diphtheriae by culture

The bacteriological culture is essential for confirming diphtheria. The following should be considered:

- A clinical specimen for culture should be obtained as soon as possible when diphtheria (involving any site) is suspected, even if treatment with antibiotics has already begun.
- Specimens should be taken from the nose and throat, and from the diphtheritic membrane. If possible, swabs also should be taken from beneath the membrane.
- **The laboratory should be alerted to the suspicion of diphtheria because isolation of *C. diphtheriae* requires special culture media containing tellurite.**
- Isolation of *C. diphtheriae* from close contacts may confirm the diagnosis of the case, even if the patient's culture is negative.

All suspected cases and their close contacts should supply specimens from the nose and throat (i.e., both a nasopharyngeal and a pharyngeal swab) for culture.

Biotype testing

After *C. diphtheriae* has been isolated, the **biotype (substrain)** should be determined. The four biotypes are *intermedius*, *belfanti*, *mitis*, and *gravis*.

Toxigenicity testing

Also, toxigenicity testing using the **Elek test** should be performed to determine if the *C. diphtheriae* isolate produces toxin. These tests are not readily available in many clinical microbiology laboratories; isolates should be sent to a reference laboratory proficient in performing the tests.

Polymerase chain reaction (PCR) testing

Additional clinical specimens for PCR testing at CDC should be collected at the time when specimens are being collected for culture. Because isolation of *C. diphtheriae* is not always possible (many patients have already received several days of antibiotics by the time a diphtheria diagnosis is considered), PCR test can provide additional supportive evidence for the diagnosis of diphtheria. The PCR assay allows for detection of the regulatory gene for toxin production (*dtxR*) and the diphtheria toxin gene (*tox*).[4] Clinical specimens (swabs, pieces of membrane, biopsy tissue) can be transported to CDC with cold packs in a sterile empty container or in silica gel sachets. For detailed information on specimen collection and shipping and to arrange for PCR testing, the state health department may contact the CDC Diphtheria Laboratory at 404-639-1730 or 404-639-1231.

Serologic testing

Measurement of the patient's serum antibodies to diphtheria toxin before administration of antitoxin may help in assessing the probability of the diagnosis of diphtheria. The state health department or CDC can provide information on laboratories that offer this test (few laboratories have the capability to accurately test antibody levels). If antibody levels are low, diphtheria cannot be ruled out accurately, but if levels are high, *C. diphtheriae* is less likely to produce serious illness.

Submission of C. diphtheriae isolates

All isolates of *C. diphtheriae*, from any body site (respiratory or cutaneous), whether toxigenic or nontoxigenic, should be sent to the CDC Diphtheria Laboratory for reference testing. Clinical specimens should also be sent to the CDC Diphtheria Laboratory for PCR testing. To arrange specimen shipping, contact your state health department.

B. *Haemophilus influenzae* type b (Hib) invasive disease (see Chapter 2)

Culture

Confirming a case of Hib requires culturing and isolating the bacterium from a normally sterile body site. Most hospital and commercial microbiologic laboratories have the ability to isolate *H. influenzae* from cultured specimens. Normally sterile site specimens for isolation of invasive *H. influenzae* include cerebrospinal fluid (CSF), blood, joint fluid, pleural effusion, pericardial effusion, peritoneal fluid, subcutaneous tissue fluid, placenta, and amniotic fluid. All Hi isolates should be also tested for antimicrobial susceptibility.

Serotype testing (serotyping)

Serotyping distinguishes encapsulated strains, including Hib, from unencapsulated (non-typeable) strains, which cannot be typed. The six encapsulated strains (designated a–f) have distinct capsular polysaccharides that can be differentiated by slide agglutination with specific antisera.

To monitor the occurrence of invasive Hib disease, microbiology laboratories should perform serotype testing of all *H. influenzae* isolates,[5,6] particularly those obtained from children < 5 years of age. To monitor the disease burden and long-term vaccine effectiveness, Hi isolates from children 5–14 years should also be serotyped and reported. Even though Hib disease has declined, laboratories need to continue routine serotype testing. Contact your state health department if serotyping is not available at your laboratory. In addition, because of inconsistencies in serotype results of Hi isolates, the CDC Meningitis and Special Pathogens Laboratory will serotype (or retest to confirm the reported serotype) all *H. influenzae* isolates from invasive disease cases among children aged < 15 years.[7, 8] Contact the laboratory at 404-639-3158 for more information.

Antigen detection

Because the type b capsular antigen can be detected in body fluids including urine, blood and CSF of patients, clinicians often request a rapid antigen detection test for diagnosis of Hib disease. Antigen detection may be used as an adjunct to culture, particularly in the diagnosis of patients who have received antimicrobial agents before specimens are obtained for culture. Methods for antigen detection include latex agglutination (LA) and counterimmunoeletro-phoresis. LA is a rapid and sensitive method used to detect Hib capsular polysaccharide antigen in CSF, serum, urine, pleural fluid, or joint fluid; Counterimmunoelectrophoresis is a more specific but less sensitive test than LA, but takes longer and is more difficult to perform.

If the Hib antigen is detected in the CSF and there is not a positive result from culture or sterile site, the patient should be considered a **probable case** of Hib disease and reported as such. Because antigen detection tests can be positive in urine and serum of persons without invasive Hib disease, persons who are identified exclusively by positive antigen tests in urine or serum should not be reported as cases. PCR assays for Hib in clinical specimens are available for research purposes only.[9] Isolation of the bacterium is needed to confirm Hi invasive disease and to test for antimicrobial susceptibility.

Subtyping

Although not widely available, subtyping the Hib bacterium on the basis of outer membrane proteins, lipopolysaccharides, enzyme electrophoresis, or pulsed-gel electrophoresis on DNA[10] can be performed for epidemiologic purposes. The state health department may direct questions about subtyping to the CDC Meningitis and Special Pathogens Laboratory at 404-639-3158.

C. Hepatitis A (see Chapter 3)

Diagnostic tests used to confirm hepatitis A infection include **serologic testing**, and **occasionally, polymerase chain reaction (PCR)-based assays** to amplify and sequence viral genomes.

Serologic testing
The diagnosis of acute hepatitis due to hepatitis A virus (HAV) is confirmed during the acute or early convalescent phase of infection by the presence of **IgM anti-HAV** in serum.

Serum for IgM anti-HAV testing should be drawn as soon as possible after onset of symptoms, since IgM anti-HAV generally disappears within 6 months after onset of symptoms.

IgG anti-HAV appears in the convalescent phase of infection, remains for the lifetime of the person, and confers enduring protection against disease.

The antibody test for **total anti-HAV** measures both IgG anti-HAV and IgM anti-HAV. The presence of total anti-HAV and absence of IgM anti-HAV indicates immunity consistent with either past infection or vaccination. Commercial diagnostic tests are widely available for the detection of IgM and total (IgM and IgG) anti-HAV in serum.

CDC Laboratory special studies
Occasionally, molecular virologic methods such as polymerase chain reaction (PCR)-based assays are used to amplify and sequence viral genomes. These assays may be helpful to investigate common source outbreaks of hepatitis A. Providers with questions about molecular virologic methods should consult with their state health department or the Division of Viral Hepatitis, Laboratory Branch, CDC.

D. Hepatitis B (see Chapter 4)

Diagnostic tests used to confirm hepatitis B infection include **serologic testing**, **subtyping (in outbreak investigations)**, and **occasionally polymerase chain reaction (PCR)-based assays** to amplify and sequence viral genomes.

Serologic Testing
The diagnosis of hepatitis B infection can be serologically confirmed either by a positive test for **IgM antibody to hepatitis B core antigen (anti-HBc)** or by a positive test for **hepatitis B surface antigen (HBsAg)** with a negative test for hepatitis A antibody (anti-HAV) (see Table 5).

Several well-defined antigen-antibody systems are associated with HBV infection, including HBsAg and anti-HBs; hepatitis B core antigen (HBcAg) and antibody to HBcAg (anti-HBc); and hepatitis B *e* antigen (HBeAg) and antibody to HBeAg (anti-HBe). Serologic assays are commercially available for all of these except HBcAg because no free HBcAg circulates in blood.

The presence of **HBsAg** is indicative of ongoing HBV infection and potential infectiousness. In newly infected persons, HBsAg is present in serum 30–60 days after exposure to HBV and persists for variable periods. Anti-HBc develops in all HBV infections, appearing at onset of symptoms or liver test abnormalities in acute HBV infection, rising rapidly to high levels, and persisting for life. Acute or recently acquired infection can be distinguished by presence of the immunoglobulin M (IgM) class of anti-HBc, which persists for approximately 6 months. However, among infected infants, passively transferred maternal anti-HBc may persist beyond the age of 12 months, and IgM anti-HBc may not be present in newly infected children < 2 years of age, especially if they acquired their infection through perinatal transmission.

In persons who recover from HBV infection, HBsAg is eliminated from the blood, usually in 2–3 months, and anti-HBs develops during convalescence. The presence of anti-HBs indicates immunity from HBV infection. After recovery from natural infection, most persons will be positive for both anti-HBs and anti-HBc, whereas only anti-HBs develops in persons who are successfully vaccinated against hepatitis B. Persons who do not recover from HBV infection and become chronically infected remain positive for HBsAg (and anti-HBc), although a small proportion (0.3% per year) eventually clear HBsAg and might develop anti-HBs.

Subtyping

Subtyping of HBsAg has occasionally been used to investigate outbreaks of hepatitis B, but this procedure is not routinely available in commercial laboratories.

CDC Laboratory special studies

Occasionally molecular virologic methods such as **polymerase chain reaction (PCR)-based assays** are used to amplify and sequence viral genomes. In conjunction with epidemiologic studies, these assays may be helpful for investigating common source outbreaks of hepatitis B. In addition, these assays are essential for detecting the emergence of vaccine-resistant strains. For example, the detection of HBV variants or "escape mutants" among vaccinated infants of HBsAg-positive women is important to determine their potential role in vaccine failures.[11] Health care professionals with questions about molecular virologic methods or those who identify HBsAg-positive events among vaccinated persons should consult with their state health department or the Epidemiology Branch, Division of Viral Hepatitis, CDC, 404-371-5910.

E. Influenza (see Chapter 5)

Methods available for the diagnosis of influenza include **virus isolation** (standard methods and rapid culture assays), **detection of viral antigens** (enzyme immunoassays [EIA], immunoflourescent antibody [IFA], and less frequently electron microscopy), **molecular detection** (polymerase chain reaction [PCR]), and **serologic testing**.

Virus isolation

Virus isolation is the gold standard for influenza diagnosis.

- Appropriate samples include nasal washes, nasopharyngeal aspirates, nasal and throat swabs, transtracheal aspirates, and bronchoalveolar lavage.

- Samples should be taken within 72 hours of onset of illness to maximize the probability of isolating virus.

- Rapid culture assays that detect viral antigens in cell culture are available. These assays can provide results in 18–40 hours as compared with an average of 4.5 days to obtain positive results from standard culture.

Direct antigen detection methods

When direct antigen detection methods are used to screen for influenza, it is important to save an aliquot of the clinical sample for further testing. These aliquots may be used for culture confirmation of direct test results and for subtyping influenza A isolates by the state public health laboratory. Full antigenic characterization of the viral isolate may be performed by the U.S. World Health Organization (WHO) Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Influenza Branch, CDC. Full characterization of a sample of isolates is necessary for the detection and tracking of antigenic variants, an essential step for selecting influenza vaccine strains.

Viral antigen detection

Viral antigen detection methods can be used to diagnose influenza infection directly from clinical material.

- Cells from the clinical sample can be stained using an immunofluorescent antibody to look for the presence of viral antigen. Nasal washes, nasopharyngeal aspirates, nasal and throat swabs, gargling fluid, transtracheal aspirates, and bronchoalveolar lavage are suitable clinical specimens.

- Commercially available kits to test for the presence of viral antigens fall into three groups; the first detects only influenza type A viruses, while the second detects both influenza type A and B viruses but does not differentiate between virus types, and the third detects both influenza type A and B viruses and distinguishes between the two. Results of these rapid antigen detection tests can be available in less than 1 hour.

- Other less frequently used methods include immunostaining and visualization of viral antigens by electron microscopy.

Molecular detection

Molecular methods can be used to detect the presence of influenza virus in a clinical specimen and to characterize the virus. These methods include detection of viral RNA by **molecular hybridization** and **reverse transcription PCR**.

- When direct antigen detection methods are used for the diagnosis of influenza, it is important to collect and reserve an aliquot of the clinical sample for possible further testing. Reserved samples may be used to confirm direct test results by culture and to subtype influenza A isolates.

- The media used to store the specimen for some rapid testing methods is inappropriate for viral culture; in this case, it is necessary to collect two separate samples.

- Full antigenic characterization of the virus may be performed by the U.S. World Health Organization (WHO) Collaborating Center for Surveillance, Epidemiology, and Control of Influenza, Influenza Branch, CDC.

Serologic testing

Serologic diagnosis of influenza infection requires paired serum specimens. The acute sample should be collected within 1 week of the onset of illness and the convalescent sample should be collected approximately 2–3 weeks later.

- **Hemagglutination inhibition (HI) tests** are the preferred method of serodiagnosis. A positive result is a four-fold or greater rise in titer between the acute and convalescent samples when tested at the same time. Serologic test results are usually available in 24 hours.

- While serologic testing can be useful in certain situations where viral culture is not possible or in special studies, serologic diagnosis of influenza is not used for national surveillance due to a lack of standardized testing methods and interpretation.

F. Measles (see Chapter 6)

Serologic testing

Serologic testing for antibodies to measles is widely available. Generally, in a previously susceptible person exposed to either vaccine- or wild-type measles virus, the IgM response starts first around the time of rash onset and is transient, persisting 1–2 months. The IgG response starts more slowly, at about 7 days after rash onset, but typically persists for a lifetime. The diagnosis of acute measles infection can be made by detecting IgM antibody to measles in a single serum specimen or by detecting a rise in the titer of IgG antibody in two serum specimens drawn roughly two weeks apart. Uninfected persons are IgM negative and will either be IgG negative or IgG positive depending upon their previous infection or vaccination histories.

Recommendations for serologic test for measles

- An enzyme immunoassay (EIA) test for IgM antibody to measles in a single serum specimen, drawn at the first contact with the suspected measles case, is the recommended method for diagnosing acute measles.

- A single specimen test for IgG is the most commonly used test for immunity to measles because IgG antibody is long lasting.

- Testing for IgG along with IgM is recommended for suspected measles cases.

- Paired sera (acute and convalescent) may be tested for a rise in IgG antibody to measles to confirm acute measles infection.

- When a patient with suspected measles has been recently vaccinated (6-45 days prior to testing) neither IgM nor IgG antibody responses can distinguish measles disease from the response to vaccination.

Tests for IgM antibody

Although there are multiple possible methods for testing for IgM antibody, enzyme immunoassays (EIAs) are the most consistently accurate tests and are therefore the recommended method. There are two formats for IgM tests. The first and most widely available is the indirect format; IgM tests based on the indirect format require a specific step to remove IgG antibodies. Problems with removal of IgG antibodies can lead to false-positive tests[12] or, less commonly, false-negative results.

The second format, IgM capture, does not require the removal of IgG antibodies. CDC has developed a capture IgM test for measles, and trained personnel from every state public health laboratory. This is the preferred reference test for measles. One direct capture IgM EIA is commercially available.

EIA tests for measles are often positive on the day of rash onset. However, in the first 72 hours after rash onset, up to 30% of tests for IgM may give false-negative results. Tests that are negative in the first 72 hours after rash onset should be repeated (**Table 1**); serum should be obtained for repeat testing 72 hours after rash onset. IgM is detectable for at least 28 days after rash onset and frequently longer.[13]

When a laboratory IgM test is suspected of being false positive (**Table 1**), additional tests may be performed. False positive IgM results for measles may be due to the presence of rheumatoid factor in serum specimens. Serum specimens from patients with other rash illness, such as Parvovirus B19, rubella, and roseola have been observed to result in false positive reactions in some IgM tests for measles. False positive tests may be suspected when thorough surveillance reveals no source or spread cases, when the case does not meet the clinical case definition, or when the IgG result is positive within 7 days of rash onset. In these situations, confirmatory tests may be done at the state public health laboratory or at CDC. IgM results by tests other than EIA can be validated with EIA tests. Indirect EIA tests may be validated with capture EIA tests.

Tests for IgG antibody

Because tests for IgG require two serum specimens and a confirmed diagnosis cannot be made until the second specimen is obtained, IgM tests are generally preferred. However, if the IgM tests remain inconclusive, a second (convalescent) serum specimen, collected 14-30 days after the first (acute) specimen, can be used to test for an increase in the IgG titer. These tests can be performed in the state laboratory or at CDC. A variety of tests for IgG antibodies to measles are available and include EIA, hemagglutination inhibition, indirect fluorescent antibody tests, and plaque reduction neutralization.

Complement fixation, although widely used in the past, is no longer recommended. The "gold standard" test for serologic evidence of recent measles virus infection is plaque reduction neutralization test of IgG in acute and convalescent paired sera.

IgG testing for laboratory confirmation of measles requires the demonstration of a rise in the titer of antibody against measles. The tests for IgG antibody should be conducted on both acute and convalescent specimens at the same time. The same type of test should be used on both specimens. The specific criteria for documenting an increase in titer depend on the test. EIA values are not titers and increases in EIA values do not directly correspond to titer rises.

Virus isolation

Isolation of measles virus in culture or detection of measles virus by reverse transcription polymerase chain reaction (RT-PCR) in clinical specimens confirms the diagnosis of measles. However, a negative culture or RT-PCR does not rule out measles because the tests are not very sensitive and are much affected by the timing of specimen collection and the quality and handling of the clinical specimens. Since culture and RT-PCR and take weeks to perform, they are rarely useful in confirming the diagnosis of measles. If positive, these tests can be useful adjuncts to diagnosing acute measles when serology results are inconclusive. Also, if measles virus is cultured or detected by RT-PCR, the viral genotype can be used to distinguish between measles disease, caused by a wild-type measles virus, and a response to measles vaccination, caused by a vaccine strain.

Although rarely useful to diagnose measles, viral culture and RT-PCR are extremely important for molecular epidemiologic surveillance to help determine 1) the origin of the virus, 2) which viral strains are circulating in the United States, and 3) whether these viral strains have become endemic in the United States. Isolation of measles virus is technically difficult and is generally performed in research laboratories.

Specimens (urine, nasopharyngeal aspirates, heparinized blood, or throat swabs) for virus culture should be obtained from clinically suspected cases of measles.

Specimens (urine, nasopharyngeal aspirates, heparinized blood, or throat swabs) for virus culture obtained from clinically suspected cases of measles should be shipped to the state public health laboratory or to the CDC at the direction of the state health department as soon as measles is confirmed. Specimens should be properly stored while awaiting case confirmation (see **Appendix 6**). Clinical specimens for virus isolation should be collected at the same time as samples taken for serologic testing. Because virus is more likely to be isolated when the specimens are collected within 3 days of rash onset, collection of specimens for virus isolation should not be delayed until laboratory confirmation is obtained. Clinical specimens should ideally be obtained within 7 days of rash onset and should not be collected if the opportunity to collect a specimen is more than 10 days after rash onset.

G. Mumps (see Chapter 7)

Acute mumps infection can be confirmed by the presence of serum mumps IgM, a significant rise in IgG antibody titer in acute and convalescent serum specimens, positive mumps virus culture, or detection of virus by RT-PCR.

Sera should be collected as soon as possible after onset of parotitis for IgM testing or as the acute specimen for examining seroconversion. The convalescent specimen for IgG detection should be drawn about 2 weeks later. IgM antibodies are detectable within the first few days of illness, reach a maximum level about a week after onset of symptoms, and remain elevated for several weeks or months.[14,15] Virus may be isolated from the buccal mucosa from 7 days before until 9 days after salivary enlargement, and from urine during the period from 6 days before to 15 days after the onset of parotitis.[16]

Immunity to mumps may be documented by the presence of serum IgG mumps-specific antibodies by EIA.

Serologic testing

The serologic tests available for laboratory confirmation of mumps acute infections and immunity vary among laboratories. The health department can provide guidance in available laboratory services and preferred tests.

- **Enzyme immunoassay (EIA).** EIA is a highly specific test for diagnosing acute mumps infection and mumps immunity. At present, there are no FDA-approved EIA tests for detection of mumps IgM antibodies. At the direction of the state health department, health-care providers and state and local health departments may send serum specimens from patients in whom the diagnosis of mumps is suspected to the CDC Measles Virus Section for IgM detection by EIA.

- **Complement fixation (CF).** Although CF tests are useful in detecting certain mumps antigens, they are not reliable for determining mumps immunity and should not be used for screening purposes.[16]

- **Hemagglutination inhibition test (HI).** As in the case of CF tests, HI tests cannot be used to assess immunity to mumps and should not be used for screening purposes. A rise in mumps HI titer can be used to diagnose mumps infection, but anamnestic responses may occur during parainfluenza infections.[16]

Viral cultures

Mumps virus can be isolated from throat swabs, urine, and cerebrospinal fluid (CSF). Efforts should be made to obtain the specimen as soon as possible after parotitis or meningitis onset. Because there are few laboratories that perform mumps virus culture, it is rarely used for clinical diagnosis in uncomplicated cases. Successful isolation should always be confirmed by immunofluorescence with a mumps-specific monoclonal antibody or by molecular techniques. Molecular typing of virus isolates provides epidemiologically important information and is now recommended (see below).

Molecular typing

Molecular techniques such as RT-PCR can be used to detect mumps RNA in appropriately collected throat swabs, urine samples, and CSF.

Molecular epidemiologic surveillance makes it possible to build a sequence database that will help track transmission pathways of mumps strains circulating in the U.S. In addition, typing methods are available to distinguish wild-type mumps virus from vaccine virus. Specimens for molecular typing should be obtained from the buccal mucosa with nasopharyngeal swabs and from urine as soon as possible after the onset of parotitis, from the day of onset to 3 days later. Specific instructions for specimen collection and shipping may be obtained from the CDC by contacting the Viral Vaccine Preventable Diseases Branch, National Immunization Program, 404-639-8230. Specimens for virus isolation and molecular typing should be sent to CDC as directed by the state health department.

H. Pertussis (see Chapter 8)

Culture

The standard and preferred laboratory test for diagnosis of pertussis is isolation of *B. pertussis* by bacterial culture.

Isolation of the *B. pertussis* bacterium is required to test for antimicrobial resistance and for molecular typing by pulse-field gel electrophoresis (PFGE). Although bacterial culture is specific for the diagnosis, it is relatively insensitive. Under optimal conditions 80% of suspected cases in outbreak investigations can be confirmed by culture; in most clinical situations isolation rates are much lower.[17] The timing of specimen collection can affect the isolation rate, as can inadequately collected specimens and concurrent use of effective antimicrobials. Because patients can remain culture positive even while taking effective antibiotics (e.g., strains that are resistant to the antibiotic), nasopharyngeal swab for culture should be obtained regardless of concurrent use of an antibiotic.

Fastidious growth requirements make *B. pertussis* difficult to isolate. Isolation of the organism using direct plating is most successful during the catarrhal stage (i.e., first 1–2 weeks of cough). All suspected cases of pertussis should have a nasopharyngeal aspirate or swab obtained from the posterior nasopharynx for culture. For *B. pertussis*, nasopharyngeal aspirates have similar or higher rates of recovery than nasopharyngeal swabs;[17-20] throat and anterior nasal swabs have unacceptably low rates of recovery of *B. pertussis*. Therefore, **specimens from the posterior nasopharynx (Figure 1), not the throat, should be obtained using Dacron® or calcium alginate swabs, not cotton, and should be plated directly onto selective culture medium or placed in transport medium.** Regan-Lowe agar or freshly prepared Bordet-Gengou medium generally is used for culture; half-strength Regan-Lowe can be used as the transport medium. Success in isolating the organism declines with prior antibiotic therapy effective against susceptible *B. pertussis* (erythromycin or trimethoprim-sulfamethoxazole), delay in specimen collection beyond the first 2 weeks of illness, or in vaccinated individuals. A positive culture for *B. pertussis* confirms the diagnosis of pertussis. For this reason, access to a microbiology laboratory that is prepared to perform this service for no cost or for limited cost to the patient is a key component of pertussis surveillance.

Polymerase chain reaction (PCR)

PCR testing of nasopharyngeal swabs or aspirates can be a rapid, sensitive, and specific method for diagnosing pertussis.[21] However, false positive results may be obtained due to contamination in the laboratory or during specimen collection.[21,22] PCR currently is available in some laboratories; the assay varies among laboratories and is not standardized. Direct comparison with culture is necessary for validation. Even if a laboratory has validated its PCR method, the result should be considered presumptive and isolation of *B. pertussis* by culture should be attempted to assure that the disease is truly pertussis. *B. pertussis* isolates can then be evaluated for erythromycin susceptibility and by PFGE. PFGE can help define the molecular epidemiology of strains circulating in the U.S.

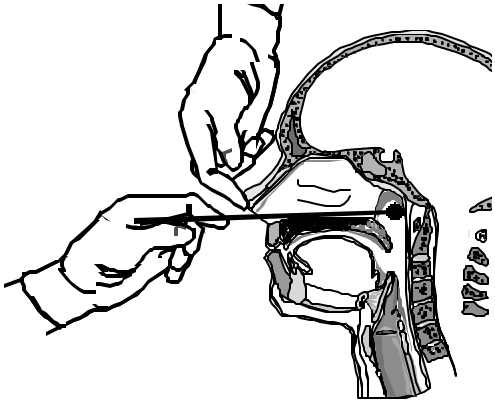

Figure 1: Proper technique for obtaining a nasopharyngeal specimen for isolation of *B. pertussis*

Serologic testing

Although serological testing has proven useful in clinical studies, it is not yet standardized. Due to lack of association between antibody levels and immunity to pertussis, results of serologic testing are difficult to interpret. For these reasons, serologic testing is not widely available. In Massachusetts, serologic testing is used for clinical diagnosis and reporting.[23] Elsewhere, with few exceptions, it is unknown if serologic testing has been appropriately validated or standardized. Therefore, serologic testing should not be relied upon to confirm cases for the purpose of national reporting. Cases meeting the clinical case definition that are serologically positive, but not culture positive or PCR positive, should be reported as probable cases.

Direct fluorescent antibody (DFA) testing

DFA testing of nasopharyngeal secretions may be useful as a screening test for pertussis. A positive DFA result may increase the probability that the patient has pertussis, but it has limited specificity (frequent false-positive results) and is not a confirmatory test. A monoclonal DFA test is available (Accu-Mab™, Biotex Laboratories, Inc., Edmonton, Canada) but the sensitivity and specificity is variable.

Elevated white blood-cell (WBC) count

An elevated WBC with a lymphocytosis (i.e., increase in lymphocyte count) is usually present in cases of pertussis. The absolute lymphocyte count can reach ≥ 20,000/mm. However, there may be no lymphocytosis in very young infants, vaccinated children, or in mild cases of pertussis among adults. The white blood-cell count is not a confirmation test.

Pulsed-field gel electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) is a type of DNA fingerprinting. This technique has been a useful tool to distinguish epidemiologically-related strains (e.g., strains from the same household or small community), while showing diversity within larger geographic areas such as cities, counties, and states.[24,25]

Questions about performing PFGE on *B. pertussis* isolates, as well as questions about isolating *B. pertussis*, performing erythromycin susceptibility, and performing PCR can be directed to the CDC Epidemic Investigations Laboratory. Call Dr. Gary Sanden at 404-639-3024 or Ms. Pam Cassiday at 404-639-1231. If needed, *B. pertussis* isolates can be sent to:

CDC, Epidemic Investigations Laboratory
Pertussis
Attention: Pam Cassiday
Building 17, Room 2227
1600 Clifton Road NE
Atlanta, GA 30333

I. Pneumococcal infection (see Chapter 9)

Culture

Diagnosis of pneumococcal infection is confirmed by culture of *S. pneumoniae* from a normally sterile body site (e.g., blood, CSF, pleural fluid, or peritoneal fluid).

Drug resistance

Based on recommendations from the National Committee for Clinical Laboratory Standards (NCCLS), clinical laboratories should test all isolates of *S. pneumoniae* from CSF for resistance to penicillin, cefotaxime or ceftriaxone, meropenem, and vancomycin.[26] For organisms from other sources, laboratories should consider testing for resistance to erythromycin, penicillin, trimethoprim-sulfamethoxazole, clindamycin, cefepime, cefotaxime or ceftriaxone, a

fluoroquinolone, meropenem, tetracycline, and vancomycin. Pneumococci resistant to vancomycin have never been described; a strain with a minimum inhibitory concentration of ≥ 2 µg/ml or zone diameter < 17 mm should be submitted to a reference laboratory for confirmatory testing, and if resistant, reported to the state health department. Because pneumococci are fastidious organisms, some susceptibility testing methods used for other organisms are not appropriate for pneumococci; see the NCCLS document for testing recommendations.[26]

J. Poliomyelitis (see Chapter 10)

Virus isolation

The likelihood of poliovirus isolation is highest from stool specimens, intermediate from pharyngeal swabs, and very low from blood or spinal fluid. The isolation of poliovirus from stool specimens contributes to the diagnostic evaluation but does not constitute proof of a causal association of such viruses with paralytic poliomyelitis.[27] Isolation of virus from the cerebrospinal fluid (CSF) is diagnostic but is rarely accomplished. To increase the probability of poliovirus isolation, at least two stool specimens and two throat swabs should be obtained 24 hours apart from patients with suspected poliomyelitis as early in the course of the disease as possible (i.e., immediately after poliomyelitis is considered as a possible differential diagnosis), but ideally within the first 15 days after onset of paralytic disease. Specimens should be sent to the state or other reference laboratories for primary isolation. Laboratories should forward isolates to CDC for intratypic differentiation to determine whether the poliovirus isolate is wild or vaccine-derived.

Isolation of wild poliovirus constitutes a public health emergency, and appropriate control efforts must be initiated immediately (in consultation among health-care providers, the state and local health departments, and CDC).

Serologic testing

Serology may be helpful in supporting or ruling out the diagnosis of paralytic poliomyelitis. An acute serum specimen should be obtained as early in the course of disease as possible, and a convalescent specimen should be obtained at least 3 weeks later. A four-fold titer rise between the acute and convalescent specimens suggests poliovirus infection. Non-detectable antibody titers in both specimens may help rule out poliomyelitis but may be falsely negative in immunocompromised persons, who are also at highest risk for paralytic poliomyelitis. In addition, neutralizing antibodies appear early and may be at high levels by the time the patient is hospitalized; thus, a four-fold rise may not be demonstrated. Vaccinated individuals would also be expected to have measurable titers, therefore vaccination history is important for serology interpretation. One of the limitations of serology is the inability to distinguish between antibody induced by vaccine-related poliovirus and antibody induced by wild virus. Serologic assays to detect anti-poliovirus antibodies are available in most commercial and state public health laboratories.

K. Rubella (see Chapter 11)

Diagnostic tests used to confirm acute or recent rubella infection or CRS include **serologic testing** and **virus isolation**.

Serologic testing

Sera should be collected as early as possible (within 7–10 days) after onset of illness, and again at least 7–14 days (preferably 2–3 weeks) later. IgM antibodies may not be detectable before day 5 after rash onset. In case of a negative rubella IgM and IgG in specimens taken before day 5, repeat serologic testing. Virus may be isolated from 1 week before to 2 weeks after rash onset. However, maximum viral shedding is up to day 4 after rash onset.

False-positive serum rubella **IgM tests** have occurred in persons with parvovirus infections or positive heterophile test (indicating infectious mononucleosis), or with a positive rheumatoid factor (indicating rheumatologic disease).[28,29] When a false-positive rubella IgM is considered, a rheumatoid factor, parvovirus IgM, and heterophile test should be done to rule out a false-positive rubella IgM test result.

The serologic tests available for laboratory confirmation of rubella infections and immunity vary among laboratories. The following tests are widely available and may be used for screening for rubella immunity and/or laboratory confirmation of disease. The state health department can provide guidance on available laboratory services and preferred tests.

Enzyme immunoassay (EIA). Most of the diagnostic testing done for rubella antibodies use some variation of the EIA, which is sensitive, widely available, and relatively easy to perform. EIA is the preferred testing method for IgM, using the capture technique; indirect assays are also acceptable.

Hemagglutination inhibition (HI) test. This once was the gold standard and most commonly used technique and allows for either screening or diagnosis (if paired acute and convalescent sera are tested). A four-fold rise or greater in HI antibody titer in paired sera is diagnostic of recent infection. The test may be modified to detect rubella-specific IgM antibody indicative of primary infection.

Latex agglutination (LA) test. The 15-minute LA test appears to be sensitive and specific for screening when performed by experienced laboratory personnel.

Immunofluorescent antibody (IFA) assay. IFA is a rapid and sensitive assay. Commercial assays for both IgG and IgM are available in the United States. Care must be taken with the IgM assay to avoid false-positive results due to complexes with rheumatoid antibody.

Virus isolation

Rubella virus can be isolated from nasal, blood, throat, urine, and cerebrospinal fluid specimens from rubella and CRS cases. The best results come from throat swabs. Efforts should be made to obtain clinical specimens for virus isolation from all cases (or from at least some cases in each outbreak) at the time of the

initial investigation. Virus may be isolated from 1 week before to 2 weeks after rash onset. However, maximum viral shedding is up to day 4 after rash onset.

Molecular typing

Rubella virus isolates are very important for surveillance. Molecular epidemiologic surveillance provides important information on:

- Origin of the virus
- Virus strains circulating in the U.S.
- Whether these strains have become endemic in the U.S.

In obtaining specimens for rubella molecular typing, collect throat swabs within 4 days of rash onset. Specimens for molecular typing from CRS cases should be collected as soon as possible after diagnosis. Appropriate specimens from CRS cases for molecular typing include throat swabs, cerebrospinal fluid, and cataracts from surgery. Strains for virus isolation should be sent to CDC for molecular typing as directed by the state health department.

Reverse transcription polymerase chain reaction (RT- PCR)

In the United Kingdom, there has been extensive evaluation of RT-PCR for detection of rubella virus in clinical specimens, documenting its usefulness.[30,31] Clinical specimens obtained for virus isolation and sent to CDC are routinely screened by RT-PCR.

L. Congenital rubella syndrome (CRS) (see Chapter 12)

Diagnostic tests used to confirm CRS include serologic assays and isolation of the virus. Laboratory confirmation can be obtained by any of the following:

- Demonstration of rubella-specific IgM antibodies in the infant's cord blood or sera. In infants with CRS, IgM antibody persists for at least 6-12 months. In some instances, IgM may not be detected until at least 1 month of age (thus, infants with symptoms consistent with CRS who test negative shortly after birth should be retested at 1 month of age).[32]

- Documentation of persistence of serum rubella IgG titer beyond the time expected from passive transfer of maternal IgG antibody.

- Isolation of rubella virus, which may be shed from the throat and urine for a year or longer.

- Detection of rubella virus by reverse-transcription polymerase chain reaction (RT-PCR).

M. Varicella (see Chapter 14)

Laboratory testing for varicella is **not routinely required** but is indicated to confirm the diagnosis in severe or unusual cases or to determine varicella susceptibility. As varicella is the most common disease confused with smallpox, rapid laboratory confirmation of VZV diagnosis in cases of vesicular-pustular rash illness that fall into the category of "moderate risk" for smallpox according to the CDC algorithm is required. **As disease continues to decline, laboratory confirmation will become standard practice.** Diagnostic tests used to confirm recent varicella infection include virus isolation and identification, in addition to serologic tests.

Virus isolation and identification

- **Rapid varicella zoster virus identification.** Rapid virus identification techniques are indicated for a case with severe or unusual disease to initiate specific antiviral therapy. The direct fluorescent antibody (DFA) test is the method of choice for rapid clinical diagnosis. This test is sensitive, specific, and widely available. Results are available within several hours. Specimens are best collected by unroofing a vesicle and then rubbing the base of a skin lesion, with a polyester swab, preferably a fresh fluid-filled vesicle. Crusts from lesions are also excellent specimens. Other specimen sources such as nasopharyngeal secretions, saliva, blood, urine, bronchial washings, and cerebrospinal fluid are considered less desirable sources than skin lesions since positive test results from such specimens are much less likely. Because viral proteins persist after cessation of viral replication, DFA may be positive when viral cultures are negative.

- **PCR.** PCR is a powerful technique that permits the rapid amplication of specific sequences of viral DNA that would otherwise be present in clinical specimens at concentrations well below detectable limits. Carefully designed RNA primers that target selected small stretches of viral DNA can be used to replicate small quantities of viral DNA extracted from clinical samples. If a PCR product of the expected size is produced, it is evidence that the virus was present in the lesion. This technique has been extended for VZV by amplifying pieces of varicella DNA that include a mutation in the base sequence that distinguishes the vaccine strain from wild-type varicella strains. Highly specific cutting enzymes (restriction endonucleases) can be selected that will cut the fragment from either wild-type strains or vaccine strain, but not both. This provides a convenient means for discriminating between them. More recently, it has been possible to apply these methods to real-time PCR machines that permit direct, single-step discrimination of vaccine from wild-type on the basis, e.g., of the difference in temperature at which the strands from vaccine versus wild-type DNA fragments reanneal on cooling. This type of approach has reduced the time required to identify a vaccine adverse event from two days to several hours.

- **Virus strain identification.** Strain identification can distinguish wild-type VZV from the vaccine (Oka/Merck) strain using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Such

testing is important in situations where it is important to distinguish wild-type from vaccine-type virus suspected vaccine adverse events. More recently, rapid real-time PCR methods using Light Cycler or TaqMan technology have made it possible to discriminate vaccine strain from wild-type VZV in a single tube assay requiring only a few hours. Post-vaccination situations for which specimens should be tested include 1) rash with more than 50 lesions ≥ 7 days after vaccination; 2) suspected secondary transmission of the vaccine virus; 3) herpes zoster in a vaccinated person; or 4) any serious adverse event. The National VZV Laboratory at CDC has the capacity to distinguish wild-type VZV from Oka strain using both conventional and real-time PCR methods. Call the National VZV laboratory at 404-639-0066, 404-639-3667, or email vzvlab@cdc.gov for details about the collection and submission of specimens for testing.

- **Virus culture.** The diagnosis of VZV infection may be confirmed by culture (isolation) of VZV. Although the virus is difficult to culture, virus isolation should be attempted in cases of severe disease, especially in immunocompromised persons, in order to confirm the diagnosis of varicella. Newer, more sensitive and rapid culture techniques may provide results within 2 to 3 days. Infectious VZV is usually recoverable from fluid from varicella lesions for 2 to 3 days and from zoster lesions for 7 days or longer. VZV may be cultured from other sites such as blood and CSF, especially in immunocompromised patients. Viable VZV cannot be recovered from crusted lesions.

- **Serologic testing.** For confirmation of disease a) IgM, and b) acute and convalescent IgG: Serological tests are available for IgG and IgM antibodies to VZV. Testing using commercial kits for IgM antibody is not recommended since available methods lack sensitivity and specificity; false positive IgM results are common in the presence of high IgG levels. The National VZV Laboratory at CDC has developed a reliable IgM capture assay. Call 404-639-0066, 404-639-3667, or email vzvlab@cdc.gov for details about the collection and submission of specimens for testing.

- **Testing susceptibles.** Single serological IgG tests may be used to identify the immune status of individuals whose history of varicella is negative or uncertain, and who may be candidates for varicella zoster immune globulin (VZIG) or vaccination. Paired acute and convalescent antibody tests are used in situations of mild or atypical presentation of disease when immediate therapy is not indicated and when, for clinical reasons, a confirmed diagnosis of the acute illness is important, e.g., a suspected second infection due to varicella. Recent evidence suggests that the latex agglutination method may result in false positive tests that could mistakenly categorize a susceptible person as immune; less sensitive commercial ELISAs are recommended for the purpose of screening.[33] Routine testing for varicella immunity following vaccination is not recommended.

Table 1. Contact persons for VPD surveillance laboratory support

Disease	Test name	Lab contact name	Lab contact phone	Lab contact fax	Name of lab	Notes
Diphtheria	Culture		( )	( )		
	Toxigenicity testing		( )	( )		
	PCR	Dr. Tanya Popovic	(404) 639-1730 (404) 639-4057	(404) 639-3970	CDC Diphtheria Laboratory	
	Serology (antibodies to diphtheria toxin)		( )	( )		
Haemophilus influenzae	Culture		( )	( )		
	Serotyping		( )	( )		
	Antigen detection		( )	( )		
	Subtyping		( )	( )		
Hepatitis A	IgM anti-HAV		( )	( )		
	Total anti-HAV		( )	( )		
	PCR		( )	( )		
Hepatitis B	IgM anti-HBc		( )	( )		
	HBsAg		( )	( )		
	Anti-HBs		( )	( )		
	Total anti-HBc		( )	( )		

Table 1. Contact persons for VPD surveillance laboratory support, con't.

Disease	Test name	Lab contact name	Lab contact phone	Lab contact fax	Name of lab	Notes
Influenza	Culture/viral isolation		( )	( )		
	Antigen detection		( )	( )		
	Serology		( )	( )		
Measles	IgM antibody		( )	( )		
	IgG antibody		( )	( )		
	Culture		( )	( )		
	PCR		( )	( )		
Mumps	Culture		( )	( )		
	IgM antibody		( )	( )		
	IgG antibody		( )	( )		
Pertussis	Culture		( )	( )		
	PCR		( )	( )		
Pneumococcal disease	Culture	Dr. Richard Facklam	(404) 639-1379	(404) 639-3123	CDC Streptococcus laboratory	Provide typing of single isolates of *S. Pneumoniae* only if isolate is from vaccinated patient.
	Penicillin resistance		( )	( )		

Table 1. Contact persons for VPD surveillance laboratory support, con't.

Disease	Test name	Lab contact name	Lab contact phone	Lab contact fax	Name of lab	Notes
Poliomyelitis	Culture		( )	( )		
	Intratypic differentiation	Dr. Mark Pallansch	(404) 639-3606	(404) 639-4011	CDC Polio Laboratory	
	Serology		( )	( )		
	CSF analysis		( )	( )		
Rubella	IgG antibody		( )	( )		
	IgM antibody		( )	( )		
	Culture		( )	( )		
	PCR	Dr. Teryl Frey	(404) 651-0927	( )		
	IgG antibody		( )	( )		
Congenital rubella syndrome	IgM antibody		( )	( )		
	Culture		( )	( )		
	PCR		( )	( )		
	Serology		( )	( )		
Varicella	DFA		( )	( )		
	Culture		( )	( )		
	Viral typing/ strain identification		(800) 672-6372	( )	Merck and Co., Inc.	

Table 2. Confirmatory and Other Useful Tests for the Surveillance of Vaccine-Preventable Diseases		
Disease	**Confirmatory tests**	**Other useful tests**
Diphtheria	Culture Toxigenicity testing PCR	Serology (antibodies to diphtheria toxin)
Haemophilus influenzae	Culture	Serotyping (identification of capsular type of encapsulated strains) Antigen detection Subtyping
Hepatitis A	IgM anti-HAV (positive)	Total anti-HAV (marker of immunity) PCR
Hepatitis B	IgM anti-HBc (positive); or HBsAg (positive) and IgM anti-HAV (negative)	Anti-HBs (marker of immunity); Total anti-HBc (marker of infection)
Influenza	Culture Antigen detection (EIA, IFA, EM) Serology PCR	
Measles	IgM Paired sera for IgG	Culture (for molecular epi) PCR
Mumps	Culture IgM IgG	IgG-for immunity testing
Pertussis	Culture PCR	

Table 2. Confirmatory and Other Useful Tests for the Surveillance of Vaccine-Preventable Diseases, con't.		
Disease	**Confirmatory tests**	**Other useful tests**
Pneumococcal disease	Culture	Penicillin resistance
Poliomyelitis	Culture-from stool, pharynx, or CSF	Intratypic differentiation (wild vs vaccine type) Paired serology CSF analysis
Rubella	Paired sera for IgG IgM Culture	PCR
Tetanus	There are no lab findings characteristic of tetanus	Serology to test for immunity
Varicella	Culture Serology	Viral typing/strain identification DFA

Table 3. Specimen collection for laboratory testing for VPDs

Disease	Test name	Specimens to take	Timing for specimen collection	Transport requirements	Collection requirements	Other notes
Diphtheria	Culture	Swab of nose, throat, membrane	ASAP, when diphtheria is suspected	< 24 hrs: Amies or similar transport medium ≥ 24 hrs: silica gel sachets	State health departments may call CDC diphtheria lab at 404-639-1730 or 404-639-4057	ALERT lab that diphtheria is suspected, so that tellurite-containing media will be used.
	PCR	swabs (as above), pieces of membrane, biopsy tissue	Take these specimens at same time as those for culture.	Silica gel sachet; or a sterile dry container at 4°C.	State health departments may call CDC diphtheria lab at 404-639-1730 or 404-639-4057	ALERT lab that diphtheria is suspected, so that specific PCR assay will be used.
	Toxigenicity testing (Elek test)	Isolate from culture (above)	After *C. diphtheriae* has been isolated	Transport medium such as Amies medium, or silica gel sachets	State health departments may call CDC diphtheria lab at 404-639-1730 or 404-639-4057	
	Serology (antibodies to diphtheria toxin)	Serum	Before administration of antitoxin	Frozen (-20°C)		Collect paired sera, taken 2-3 weeks apart.

Table 3. Specimen collection for laboratory testing for VPDs, con't.

Disease	Test name	Specimens to take	Timing for specimen collection	Transport requirements	Collection requirements	Other notes
***Haemophilus influenzae* type b**	Culture	Blood	ASAP	Blood culture bottles w/broth or lysis-centrifugation tube	Collect 3 separate samples in a 24-hr period.	Request that lab conduct serotyping on any *H. influenzae* isolate from any normally sterile site.
	Culture	CSF	ASAP	Sterile, screw-capped tube		
	Culture	Other normally sterile site	ASAP			
	Serotyping	Isolate from culture (above)				Highest priority are isolates from persons < 15 years.
	Antigen detection	Any normally sterile site				
Hepatitis A	IgM anti-HAV	Serum	ASAP after symptom onset (detectable up to 6 months)	All sera to be tested for serologic markers of HAV and HBV infection can be kept at ambient temperatures, refrigerated, or frozen for short term (< 48 hours). For greater than 48 hours storage, sera should be frozen or refrigerated.	Non-hemolyzed	
	Total anti-HAV	Serum	No time limit		Non-hemolyzed	Measures both IgM and IgG.

Table 3. Specimen collection for laboratory testing for VPDs, con't.						
Disease	**Test name**	**Specimens to take**	**Timing for specimen collection**	**Transport requirements**	**Collection requirements**	**Other notes**
Hepatitis B	IgM anti-HBc	Serum	ASAP after symptom onset (Detectable up to 6 months)		Non-hemolyzed	
	HBsAg	Serum			Non-hemolyzed	Positive HBsAg with negative anti-HAV confirms hepatitis B.
	Anti-HBs	Serum	1–2 months after vaccination		Non-hemolyzed	
Influenza	Culture/viral isolation	Nasal wash, nasopharyngeal aspirates, nasal/throat swabs, transtracheal aspirate, bronchoalveolar lavage	Within 72 hours of onset of illness	Transport specimens at 4^{o}C if tests are to be performed within 72 hours; otherwise, freeze at -70^{o}C until tests can be performed.		
Influenza con't. on next page	Antigen detection	Nasal wash, nasopharyngeal aspirate, nasal/throat swabs, gargling fluid, transtracheal aspirates, bronchoalveolar lavage	Within 72 hours of onset of illness	Transport specimens at 4^{o}C if tests are to be performed within 72 hours; otherwise, freeze at -70^{o}C until tests can be performed.		Save an aliquot of the clinical sample for confirmation and isolation. Viral isolates may be further characterized by WHO/CDC.

Table 3. Specimen collection for laboratory testing for VPDs, con't.

Disease	Test name	Specimens to take	Timing for specimen collection	Transport requirements	Collection requirements	Other notes
Influenza, con't.	Serology	Paired sera	Acute: within one week of onset Convalescent: 2-3 weeks after acute	Store at 4°C or frozen		Four-fold rise is a positive result. Consider vaccination history.
Measles	Culture/PCR	Urine, nasopharyngeal aspirates, heparinized blood, throat swabs	Collect at same time as samples for serology (best within 3 days of rash onset)			PCR for molecular typing. Do not collect if after 10 days from rash onset.
	IgM antibody	Serum	ASAP, and repeat 72 hours after onset if first negative			IgM is detectable for at least 28 days after rash onset.
	IgG antibody	Paired sera	Acute: ASAP after rash onset (7 days at the latest) Convalescent: 10-30 days after acute			
Mumps	Culture	Throat swabs, urine, CSF				
	IgM antibody	Serum	ASAP, antibodies peak about a week after onset			
	IgG antibody	Paired sera	Acute: within several days of onset Convalescent: 2 weeks after acute			

Table 3. Specimen collection for laboratory testing for VPDs, con't.

Disease	Test name	Specimens to take	Timing for specimen collection	Transport requirements	Collection requirements	Other notes
Pertussis	Culture	Posterior nasopharyngeal swab or aspirate	Within the first 2 weeks of cough onset	Swabs: half-strength charcoal horse blood agar at 4°C Aspirates: in catheter trap at 4°C	Use Dacron or calcium alginate (not cotton) swabs with flexible shaft or aspiration by catheter attached to catheter trap.	Inoculate selective primary isolation media such as charcoal horse blood agar or Bordet-Genou as soon as possible. Negative culture does NOT rule out pertussis.
	PCR	Nasopharyngeal swab or aspirate	Within the first 2 weeks of cough onset	Short term at 4°C; long term -20°C or below	Use Dacron or calcium alginate (not cotton) swabs with flexible shaft or aspiration by catheter attached to catheter trap.	PCR should be validated with culture when possible.
	Serology	Acute and convalescent sera	Acute: within the first 2 weeks of cough onset Convalescent: 3-9 weeks after cough onset	-20°C		Results are presumptive and should be validated with culture. Serologic results are not currently accepted as laboratory confirmation for purposes of national surveillance.
Pneumo-coccal disease	Culture	Normally sterile site				
	Penicillin resistance	Isolate from culture (above)				

Table 3. Specimen collection for laboratory testing for VPDs, con't.						
Disease	**Test name**	**Specimens to take**	**Timing for specimen collection**	**Transport requirements**	**Collection requirements**	**Other notes**
Poliomyelitis	Culture	Stool, pharyngeal swab, CSF	Acute	Sterile, screw capped container	No carrier for stool; saline buffer for swabs	Maintain frozen or transport rapidly to lab; avoid desiccation of swab specimens.
	Intratypic differentiation	Isolate from culture (above)				Maintain frozen or transport rapidly to lab; avoid desiccation of swab specimens.
	Serology	Paired sera	Acute: ASAP Convalescent: 3 weeks after acute			
Rubella	IgM antibody	Serum	Within 7–10 days of onset			
	IgG antibody	Paired sera	Acute: within 7–10 days onset Convalescent: 2–3 weeks after acute			
	Culture/PCR	Nasopharyngeal swab/wash, throat, urine, CSF	Within 4 days of onset	Viral transport media		

Table 3. Specimen collection for laboratory testing for VPDs, con't.

Disease	Test name	Specimens to take	Timing for specimen collection	Transport requirements	Collection requirements	Other notes
Congenital rubella syndrome (CRS)	IgM antibody	Serum	As soon as possible, within 6 months of birth			
	IgG antibody	Paired sera				Confirmation is by documenting persistence of serum IgG titer beyond the time expected from passive transfer of maternal IgG antibody.
	Culture/PCR	Nasopharyngeal swab/wash, urine, blood, CSF	As soon as possible; every 1–3 months until cultures are repeatedly negative	Viral transport media		
Varicella	Serology	Serum	Immune status: collect anytime except during acute illness Paired serologic diagnosis: sera-acute within 7–10 days of onset; convalescent 2–3 weeks after acute			Single IgG assay useful to assess immune status. Paired serum used to identify recent infection, but not method of choice when rapid diagnosis needed.
	Direct immuno-fluorescent antibody (DFA)	Scraping/swab from base of vesicle	Acute illness 2–3 days after rash onset and fresh vesicles			Used for rapid diagnosis.

Varicella con't. on next page

Table 3. Specimen collection for laboratory testing for VPDs, con't.						
Disease	**Test name**	**Specimens to take**	**Timing for specimen collection**	**Transport requirements**	**Collection requirements**	**Other notes**
Varicella, con't.	Culture	Fluid from vesicles, nasal or throat swabs, serum, spinal fluid, urine, bronchial tree washing or inflamed joints	Acute illness 2–3 days after rash onset and fresh vesicles			Definitive diagnosis, but not useful for rapid diagnosis.
	Viral typing/strain identification	Viral isolate (from culture)	Within 2–3 days of rash	Storage more than a few hours must be kept on dry ice or frozen at -70°C or below		Merck and Co., Inc., offers a free viral identification service using PCR analysis (1-800-672-6372).

Table 4. Interpretation of measles enzyme immunoassay results*

IgM Result	IgG Result	Previous infection history	Current infection	Comments
+	– or +	Not vaccinated, no prior history of measles	Recently received 1st dose of measles vaccine	Seroconversion. IgG response depends on timing of specimen collection
+	– or +	Not vaccinated, no prior history of measles	Wild-type measles	Seroconversion. Classic clinical measles. IgG response depends on timing of specimen collection
+	– or +	Previously vaccinated, primary vaccine failure	Recently received 2nd dose of measles vaccine	Seroconversion. IgG response depends on timing of specimen collection
–	+	Previously vaccinated, IgG+	Recently received 2nd dose of measles vaccine	IgG level may stay the same or may boost
+	+	Previously vaccinated, IgG+	Wild-type measles	May have few or no symptoms (e.g., no fever or rash).
+	+	Recently vaccinated	Exposed to wild-type measles	Cannot distinguish between vaccine or wild-type virus; evaluate on epidemiologic grounds.**
–	+	Distant history of natural measles	Vaccine	IgG level may stay the same or may boost
+ (at least in some patients)	+	Distant history of natural measles	Wild-type measles	May have few or no symptoms.

* These results are those expected when using the capture IgM and indirect IgG enzyme immunoassays and may not apply to different assays due to different techniques and sensitivities/specificities.

** However, in this circumstance, IgM testing will be helpful if negative; it could rule out wild-type measles infection (if negative).

Table 5. Interpretation of Hepatitis B Serological Tests

HBsAg	anti-HBs	anti-HBc IgM	anti-HBc IgG	Interpretations	Comments
+	-	-	-	Early acute infection	Patient is infectious; consider vaccination for susceptible household and sexual contacts.
+	-	+	+	Acute infection	Patient is infectious; consider vaccination for susceptible household and sexual contacts.
+	-	-	+	Chronic infection	Patient is infectious; patient should be evaluated for chronic liver disease; vaccinate susceptible household and sexual contacts.
-	+	+/-	+	Resolved infection	Patient is immune.
-	-	+	-	"Window period" following acute infection	Patient is not infectious.
-	-	-	+	Remote infection with loss of detectable anti-HBsAg; remote infection with possible low-level HBsAg; false positive test	Patient is non-infectious in most settings (household, sexual, needlestick exposures).
-	+	-	-	Immune following vaccination; resolved infection with loss of detectable anti-HBc	Patient is immune.

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