# Chapter 22: Laboratory Support for the Surveillance of Vaccine-Preventable Diseases

*Authors: Sandra W. Roush, MT, MPH; Bernard Beall, PhD; Lesley McGee, PhD; Pam Cassiday, MS; Michael D Bowen, PhD; Joe Icenogle, PhD; M. Steven Oberste, PhD; Daniel C. Payne, PhD, MSPH; Paul A.Rota, PhD; D. Scott Schmid, PhD; Carole Hickman, PhD; Michael Shaw, PhD; Maria Lucia Tondella, PhD; Annemarie Wasley, ScD; Xin Wang, PhD*

## I. Surveillance of Vaccine-Preventable Diseases

Surveillance for vaccine-preventable diseases (VPDs) requires the close collaboration of clinicians, public health professionals, and laboratorians. Public health surveillance relies on both clinical and laboratory reports of VPDs; therefore, appropriate specimen collection, transport, and laboratory testing are essential. This chapter provides guidelines on specimen collection for each VPD and interpretation of 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 names and contact information for 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 related information.

**Table 1. Contact persons for VPD surveillance laboratory support**

| Disease | Test name | Lab name | Lab contact/Phone |
|---|---|---|---|
| **Diphtheria** | Culture<br>Toxigenicity testing<br>PCR | CDC Pertussis and Diphtheria Laboratory | Dr. M. Lucia Tondella<br>mlt5@cdc.gov<br>404-639-1239<br>or<br>Pam Cassiday<br>pxc1@cdc.gov<br>404-639-1231<br>FAX: 404-718-2098 |
| ***Haemophilus influenzae*** | Culture<br>Real-time PCR<br>Serotyping slide agglutination<br>Molecular typing (WGS) | CDC Bacterial Meningitis Laboratory | Dr. Xin Wang<br>gqe8@cdc.gov<br>404-639-5474<br>FAX: 404-639-4421 |
| **Hepatitis A** | | Hepatitis Reference Laboratory | Saleem Kamili<br>sek6@cdc.gov<br>404-639-4431<br>FAX 404639-4431 |
| **Hepatitis B** | | Hepatitis Reference Laboratory | Saleem Kamili<br>sek6@cdc.gov<br>404-639-4431<br>FAX 404639-4431 |

| Disease | Test name | Lab name | Lab contact/Phone |
|---|---|---|---|
| **Influenza** | Culture/viral isolation<br>Antigen detection<br>RT-PCR/<br>real-time RT-PCR | Influenza Division, Surveillance and Diagnosis Branch, Diagnostic Development Team | Stephen Lindstrom, PhD<br>Team Lead,<br>Diagnostic Development Team<br>sql5@cdc.gov<br>flusupport@cdc.gov<br>404-639-1587<br>FAX: 404-639-2350 |
| **Measles** | IgM antibody<br>IgG antibody<br>Virus isolation<br>RT-PCR<br>Viral genotyping | Viral Vaccine-Preventable Diseases Branch | Dr. Paul Rota<br>par1@cdc.gov<br>404-639-4181<br>FAX: 404-639-4187 |
| **Meningococcal disease** | Culture<br>Real-time PCR<br>Serogrouping slide agglutination | CDC Bacterial Meningitis Laboratory | Dr. Xin Wang<br>gqe8@cdc.gov<br>404-639-5474<br>FAX: 404-639-4421 |
| **Mumps** | Virus isolation<br>IgM antibody<br>IgG antibody<br>RT-PCR<br>Viral genotyping | Vaccine Preventable Diseases Branch | Dr. Carole Hickman<br>cjh3@cdc.gov<br>404-639-3339<br>FAX: 404-639-4187 |
| **Pertussis** | Culture<br>PCR<br>Serology | CDC Pertussis and Diphtheria Laboratory | Dr. M. Lucia Tondella<br>mlt5@cdc.gov<br>404-639-1239<br>or<br>Pamela Cassiday<br>pc1@cdc.gov<br>404-639-1231<br>FAX: 404-718-2198 |
| **Pneumococcal disease** | Culture<br>PCR<br>Susceptibility testing<br>Serotyping (conventional or PCR-based)<br>Genotyping<br>Antibiotic | CDC Streptococcus Laboratory | Dr. Bernard Beall<br>beb0@cdc.gov<br>404-639-1237<br>or<br>Dr. Lesley McGee<br>LMcGee@cdc.gov<br>404-639-0455<br>FAX: 404-639-2070 |
| **Poliomyelitis** | Culture<br>Intratypic differentiation<br>Serology | CDC Polio/Picornavirus Laboratory | Cara Burns<br>cburns@cdc.gov<br>404-639-5499<br>FAX: 404-639-4011 |

| Disease | Test name | Lab name | Lab contact/Phone |
|---|---|---|---|
| **Rotavirus** | Antigen EIA<br>RT-PCR, qRT-PCR<br>Genotyping<br>Sequencing | CDC Rotavirus Laboratory | Dr. Michael K Bowen<br>mkb6@cdc.gov<br>404-639-4922<br>FAX: 404-639-3645 |
| **Rubella** | IgM antibody<br>IgG antibody<br>IgG Avidity<br>Culture<br>RT-PCR | CDC MMR & Herpes Virus Laboratory | Dr. Joe Icenogle<br>jicenogle@cdc.gov<br>404-639-4557<br>FAX: 404-639-1516 |
| **Congenital rubella syndrome** | IgM antibody<br>IgG antibody<br>Culture<br>RT-PCR<br>Serology | CDC MMR & Herpes Virus Laboratory | Dr. Joe Icenogle<br>jicenogle@cdc.gov<br>404-639-4557<br>FAX: 404-639-1516 |
| **Varicella** | PCR<br>DFA<br>Culture<br>Serology<br>Viral typing/strain identification | National VZV Laboratory | Dr. Scott Schmid<br>sschmid@cdc.gov<br>404-639-0066<br>FAX: 404-639-4056 |

## II. General Guidelines for Specimen Collection and Laboratory Testing

Specimen collection and shipping are important steps in obtaining laboratory diagnosis or confirmation for VPDs. Guidelines have been published for specimen collection and handling for viral and microbiologic agents.[1–3] Information is also available on using Centers for Disease Control and Prevention (CDC) laboratories as support for reference and disease surveillance;[4] this includes

- a central website (https://www.cdc.gov/laboratory/specimen-submission/index.html) for requesting lab testing;
- the form (https://www.cdc.gov/laboratory/specimen-submission/pdf/form-50-34.pdf) required for submitting specimens to CDC (see Appendix 23, Form # CDC 0.5034);
- information on general requirements for shipment of etiologic agents (Appendix 24 [http://www.cdc.gov/vaccines/pubs/surv-manual/appx/appendix24-etiologic-agent.pdf])—although written to guide specimen submission to CDC, this information may be applicable to the submission of specimens to other laboratories; and
- the CDC Infectious Diseases Laboratories Test Directory that contains not only a list (https://www.cdc.gov/laboratory/specimen-submission/list.html) of orderable tests for that institution, but also detailed information on appropriate specimen types, collection methods, specimen volume, and points of contact.

In addition, there are 4 VPD Reference Centers—public health laboratories that perform testing for 7 VPDs using standardized methods developed by CDC https://www.aphl.org/aboutAPHL/publications/Documents/ID_VPDQuickReferenceGuide_92014_updated.pdf).

## III. Disease-specific Guidelines for Specimen Collection and Laboratory Testing

This chapter provides a quick reference summary of the laboratory information from Chapters 1–17 of this manual. Confirmatory and other useful tests for surveillance of vaccine-preventable diseases are listed below in Table 2.

**Table 2. Confirmatory and other useful tests for the surveillance of vaccine-preventable diseases**

| Disease | Confirmatory tests | Other useful tests |
|---|---|---|
| **Diphtheria** | Culture<br>Toxigenicity testing | PCR<br>Serology (antibodies to diphtheria toxin)<br>MALDI-TOF |
| ***Haemophilus influenzae*** | Culture<br>RT-PCR | Serotyping slide agglutination or PCR (identification of capsular type of encapsulated strains)<br>Antigen detection<br>Molecular typing |
| **Hepatitis A**<br>**Hepatitis B** | IgM anti-HAV (positive)<br>IgM anti-HBc (acute infection)<br>HBsAg (acute or chronic infection)* | Total anti-HAV; IgG anti-HAV (markers of immunity)<br>PCR for HAV RNA (marker of current infection)<br>Anti-HBs (marker of immunity)<br>Total anti-HBc (marker of past or present infection)<br>PCR for HBV DNA (marker of current infection) |
| **Influenza** | Culture<br>Antigen detection (EIA, IFA, EM)<br>RT-PCR | |
| **Measles** | IgM<br>RT-PCR<br>Virus isolation | IgG<br>IgG for seroconversion or 4-fold titer rise<br>Avidity (case classification) |
| **Meningococcal disease** | Culture | Serogrouping<br>Slide agglutination or PCR (identification of capsular type of encapsulated strains)<br>Antigen detection<br>Molecular typing |
| **Mumps** | Virus isolation<br>RT-PCR | IgM<br>IgG for seroconversion or 4-fold titer rise (not recommended for previously vaccinated persons) |
| **Pertussis** | Culture<br>PCR | Serology |
| **Pneumococcal disease** | Culture<br>PCR | WGS-based deduction of all strain features (serotype, antimicrobial resistance, MLST genotype)<br>- serotyping<br>- PCR deduction of serotypes<br>- strain identification (MLST, PFGE) |

| Disease | Confirmatory tests | Other useful tests |
|---|---|---|
| **Poliomyelitis** | Culture-from stool, pharynx, or CSF | Intratypic differentiation (wild vs. vaccine type)<br>Paired serology<br>CSF analysis |
| **Rotavirus** | | RT-PCR/qRT-PCR |
| **Rubella** | Paired sera for IgG IgM | Culture |
| **Tetanus** | There are no lab findings characteristic of tetanus. | Serology (for immunity testing) |
| **Varicella** | PCR | DFA<br>Culture<br>Serology<br>Genotyping |

*Abbreviations: EIA, enzyme-linked immunosorbent assay; IFA, indirect fluorescent antibody; EM, electron microscopy; PCR, polymerase chain reaction; MLST, multilocus sequence typing; PFGE, pulsed field gel electrophoresis; CSF, cerebral spinal fluid; IgG, immunoglobulin G; IgM, immunoglobulin M; HAV, hepatitis A virus; HBV, hepatitis B virus; anti-HBc, hepatitis B core antibody; HBsAg, hepatitis B surface Ag; qRT-PCR, quantitative reverse transcription polymerase chain reaction*

* *Confirmation of HBsAg positive results by HBsAg neutralization assay should be performed as specified in test package insert.*

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 specimens to collect, timing of specimen collection, or where or how to transport specimens, call the state health department and the state laboratory.

### Table 3. Specimen collection for laboratory testing for VPDs

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Diphtheria** | Culture<br>Note: ALERT lab that diphtheria is suspected, so that tellurite-containing media will be used. | Swab of nose, throat, membrane | ASAP, when diphtheria is suspected | <24 hrs: Amies' or similar transport medium<br>≥24 hrs: silica gel sachets | State health departments may call CDC diphtheria lab at 404-639-1231 or 404-639-1239. |
| **Diphtheria** | PCR<br>Note: ALERT lab that diphtheria is suspected, so that specific PCR assay will be used. | Swabs (as above), pieces of membrane, biopsy tissue | Take these specimens at same time as those for culture. | Swabs, silica gel sachet; or a sterile dry container at 4°C | State health departments may call CDC diphtheria lab at 404-639-1231 or 404-639-1239. |
| **Diphtheria** | 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 404-639-1231 or 404-639-1239. |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Diphtheria** | Serology (antibodies to diphtheria toxin)<br><br>Note: Collect paired sera, taken 2–3 weeks apart.<br><br>This test is currently not available at CDC. | Serum | Before administration of antitoxin | Frozen (-20°C) | Not useful if diphtheria antitoxin was administered. |
| ***Haemophilus influenzae* type b** | Culture<br><br>Note: Request that lab conduct serotyping on any *H. influenzae* isolate from any normally sterile site. | Blood | ASAP | Blood culture bottles w/broth or lysis-centrifugation tube | Collect 3 separate samples in a 24-hr period. |
| ***Haemophilus influenzae* type b** | Culture<br><br>Note: Request that lab conduct serotyping on any *H. influenzae* isolate from any normally sterile site. | CSF<br><br>Other normally sterile site | ASAP | Sterile, screw-capped tube | |
| ***Haemophilus influenzae* type b** | Serotyping slide agglutination | culture isolate | | Chocolate slant, frozen, lyophilized or silica gel pack | Highest priority are isolates from persons <15 years. |
| ***Haemophilus influenzae* type b** | Antigen detection | CSF | ASAP | Sent frozen on blue ice packs | |
| ***Haemophilus influenzae* type b** | PCR for identification and serotyping | Any normally sterile site | ASAP | Sent frozen on blue ice packs | |
| **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 (<48 hours) for short term. For longer than 48 hours storage, sera should be frozen. | Follow standard procedures for serum separation. |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Hepatitis A** | Total anti-HAV<br>Note: Measures both IgM and IgG.<br>IgG anti-HAV | Serum | No time limit | Samples can be kept at ambient temperatures, refrigerated (<48 hours) for short term. For longer than 48 hours storage, sera should be frozen. | Follow standard procedures for serum separation. |
| **Hepatitis A** | HAV RNA by PCR | Serum | ASAP after symptom onset (detectable up to 2 weeks) | Store and ship samples frozen | Follow standard procedures for serum separation. |
| **Hepatitis B** | IgM anti-HBc | Serum | ASAP after symptom onset (detectable up to 6 months) | Samples can be kept at ambient temperatures, refrigerated (<48 hours) for short term. For storage longer than 48 hours, sera should be frozen. | Follow standard procedures for serum separation. |
| **Hepatitis B** | HBsAg<br>Note: HBsAg-positive results should be confirmed by HBsAg neutralization assay as specified in the package insert for each assay. | Serum | ASAP after symptom onset | Samples can be kept at ambient temperatures, refrigerated (<48 hours) for short term. For longer than 48 hours storage, sera should be frozen. | Follow standard procedures for serum separation. |
| **Hepatitis B** | Anti-HBs | Serum | 1–2 months after vaccination | Samples can be kept at ambient temperatures, refrigerated (<48 hours) for short term. For longer than 48 hours storage, sera should be frozen. | Follow standard procedures for serum separation. |
| **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°C if tests are to be performed within 72 hours; otherwise, freeze at -70°C until tests can be performed. | |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Influenza** | Antigen detection and RT-PCR | Nasal wash, nasopharyngeal aspirate, nasal/ throat swabs, gargling fluid, transtracheal aspirates, bronchoalveolar lavage | Within 72 hours of onset of illness | Transport specimens at 4°C if tests are to be performed within 72 hours; otherwise, freeze at -70°C until tests can be performed. | Note: Save an aliquot of the clinical sample for confirmation and isolation. Viral isolates may be further characterized by WHO/CDC. |
| **Measles** | Virus isolation<br>RT-PCR | Nasopharyngeal aspirates, throat swabs, urine, heparinized blood | Collect at same time as samples for serology (best within 3 days of rash onset) | Transport specimens at 4°C if tests are to be performed within 72 hours; otherwise, freeze at -70°C until tests can be performed. | Note: PCR for genotypingCollect up to 10 days from rash onset. |
| **Measles** | IgM antibody | Serum | ASAP after rash onset and repeat 72 hours after onset if first negative | Ship on cold pack | Note: IgM is detectable for at least 30 days after rash onset. |
| **Measles** | IgG antibody | Paired sera | Acute: ASAP after rash onset (7 days at the latest)<br>Convalescent: 14-30 days after acute | | |
| **Meningococcal disease** | Culture*<br>Note: Request that lab conduct serogrouping on any *N. meningitidis* isolate | Blood | ASAP | Blood culture bottles w/broth or lysis-centrifugation tube | . |
| **Meningococcal disease** | Culture*<br>Note: Request that lab conduct serogrouping on any *N. meningitidis* isolate | CSF<br>Other normally sterile site | ASAP | Sterile, screw-capped tube | |
| **Meningococcal disease** | Serogrouping slide agglutination | Isolate from culture (above) | | Slant, frozen, lyophilized or silica gel pack | |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Meningococcal disease** | PCR for detection and serogrouping | Any normally sterile site | ASAP | Sent frozen on blue ice packs | |
| **Meningococcal disease** | Antigen detection | Any normally sterile site | ASAP | Sent frozen on blue ice packs | |
| **Mumps** | Virus isolation<br>RT-PCR | Buccal/parotid swabs; CSF only for aseptic meningitis; urine for cases of orchitis | Ideally 0–3 days after parotitis onset but up to 11–14 days | Transport specimens at 4°C if tests are to be performed within 72 hours; otherwise, freeze at -70°C until tests can be performed. | Massage the salivary/parotid gland area for 30 seconds prior to swab collection. |
| **Mumps** | IgM antibody | Serum | Ideally ≥3 days post parotitis onset | Ship on cold pack | |
| **Mumps** | IgG antibody | Paired sera for seroconversion or 4-fold rise in titer | Acute: ideally 0–3 days post parotitis onset<br>Convalescent: 2 weeks after acute | Ship on cold pack | Four-fold rise in titer not recommended for previously vaccinated persons. |
| **Pertussis** | Culture<br>Note: Inoculate selective primary isolation media such as charcoal horse blood agar or Bordet-Gengou as soon as possible.<br>A negative culture does NOT rule out pertussis | Posterior nasopharyngeal swab or aspirate | Within the first 2 weeks of cough onset | Swabs: half-strength charcoal horse blood agar at 4°C<br>Swabs in Regan-Lowe transport<br>Aspirates: in saline in capped syringe at 4°C | Use polyester (such as Dacron), rayon or nylon nasopharyngeal swab. Flocked swabs are preffered. Shaft may be flexible plastic, aluminium, or twisted wire. Aspirates may be collected with a syringe and catheter by introducing a small amount of saline into the nasopharyngeal cavity and collecting it in the syringe. |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Pertussis** | PCR<br>Note: PCR should be validated with culture when possible. | Nasopharyngeal swab or aspirate | Within the first 2 weeks of cough onset | Swabs in Regan-Lowe transport at 4°C<br>Aspirates in saline in capped syringe at 4°C<br>For PCR testing only, swab may be placed in a sterile tube and sent at 4°C (short term storage) or -20°C or below (long term storage) | Use polyester (such as Dacron) rayon, or nylon phatyngeal swab. Flocked swabs are preferred. Shaft may be flexible plastic, aluminum, or twisted wire. Aspirates may be collected with a syringe and catheter by introducing a small amount of saline into the nasopharyngeal cavity and collecting it in the syringe. |
| **Pertussis** | Serology | Serum | 2–8 weeks of cough onset | Short term at 4°C; long-term at -20°C or below | Serologic results are currently not accepted as laboratory confirmation for purposes of national surveillance. |
| **Pneumococcal disease** | Culture | Normally sterile site | As soon as possible after onset of clinical illness but before administration of antibiotics | Blood culture bottles w/broth or lysis-centrifugation tube or, if from another sterile site, a sterile, screw-capped tube | Collect 2 separate blood samples in a 24-hr period. Most other sterile specimens (e.g., CSF) are collected only once. |
| **Pneumococcal disease** | PCR | Normally sterile site | ASAP, soon after administration of antibiotics is a viable option | Send specimen frozen on blue ice packs | PCR |
| **Pneumococcal disease** | PCR deduction of serotype | Culture-negative sterile site specimen | Specimen frozen immediately | | PCR deduction of serotype |
| **Pneumococcal disease** | Susceptibility testing | Pure culture | | Slant, frozen, or silica packet | Susceptibility testing |
| **Pneumococcal disease** | Serotyping | Pure culture | | Slant, frozen, or silica packet | Serotyping |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Poliomyelitis** | Culture | Stool, pharyngeal swab, CSF | Acute | Sterile, screw-capped container | No carrier for stool; saline buffer for swabs.<br>Note: Maintain frozen or transport rapidly to lab; avoid desiccation of swab specimens. |
| **Poliomyelitis** | Intratypic differentiation | Isolate from culture (above) | | | Note: Maintain frozen or transport rapidly to lab; avoid desiccation of swab specimens. |
| **Poliomyelitis** | Serology | Paired sera | Acute: ASAP<br>Convalescent: 3 weeks after acute | | |
| **Rotavirus gastroenteritis** | EIA, RT-PCR, qRT-PCR, genotyping, sequencing | Stool | First to fourth day of illness optimal | Sterile, screw-capped container | Bulk stool<br>Keep frozen or transport rapidly to lab on cold packs; avoid multiple freeze-thaw cycles. |
| **Rotavirus-associated seizures** | RT-PCR, qRT-PCR | CSF | ASAP after symptoms begin | Sterile, screw-capped container | No carrier.<br>Keep frozen; avoid multiple freeze-thaw cycles. |
| **Rubella** | IgM antibody | Serum | ASAP, and repeat 96 hours after onset if first negative | Maintain at 4°C and ship on ice | |
| **Rubella** | IgG antibody | Serum<br>Paired sera | Acute: ASAP after rash onset (7-days at the latest)<br>Convalescent: 14–30 days after acute | Maintain at 4°C and ship on ice | Paired sera must be run in parallel |
| **Rubella** | Culture/PCR | Nasopharyngeal swab/wash, throat swab, urine | Collect at same time as samples for serology (best within 3 days of rash onset and no later than 10 days post onset) | Viral transport media; ship frozen or on ice | Note: Maintain frozen (except urine) or transport rapidly to lab; avoid desiccation of swab specimens. |
| **Congenital rubella syndrome** | IgM antibody | Serum | As soon as possible, within 6 months of birth | Maintain at 4°C and ship on ice | |

| Disease | Test name | Specimens to take | Timing for specimen collection | Transport requirements | Collection requirements |
|---|---|---|---|---|---|
| **Congenital rubella syndrome** | IgG antibody | Serum | After 9 months of age, but before vaccination with MMR vaccine | Maintain at 4°C and ship on ice | Note: Confirmation is by documenting persistence of serum IgG titer beyond the time expected from passive transfer of maternal IgG antibody. |
| **Congenital rubella syndrome** | Culture/PCR | Nasopharyngeal swab/wash, urine, blood, cataracts | As soon as possible for confirmation; to monitor shedding in positive cases; after 3 months, every month until cultures are repeatedly negative | Viral transport media; ship frozen or on ice | Note: Maintain frozen (except urine) or transport rapidly to lab; avoid desiccation of swab specimens. |
| **Varicella** | Serology | Serum | Immune status: collect anytime except during acute illness<br>Paired serologic diagnosis: acute within 7–10 days of onset | | Single IgG assay is useful to assess immune status.<br>Paired serum distinguishes between recent primary infection and past infection. |
| **Varicella** | VZV IgG avidity | | | Sera should be shipped frozen on dry ice or refrigerated in cold packs | |
| **Varicella** | VZV PCR | 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; highly sensitive, specific and rapid |
| **Varicella** | Vaccine wild type discrimination | Material from vesicles, scabs, saliva | From day of rash onset until crusted lesions resolve | Vesicular swabs and scabs can be shipped dry at ambient temperature; saliva shipped frozen on dry ice or refrigerated on cold pack | Analysis of vaccine associated SNP using realtime PCR methods strategies; availability limited to specialized labs, e.g. CDC, Columbia University, and the Vaccine Preventable Disease Reference Centers |

* Neisseria meningitidis *culture cannot be performed on specimens sent to CDC, but CDC is available to provide advice and answer questions on culture methods.*

## A. Diphtheria

(see Chapter 1 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt01-dip.html])

Diagnostic tests used to confirm infection include isolation of *Corynebacterium diphtheriae* by culture and Elek testing of isolates for diphtheria toxin production. Although no other tests for confirmg diphtheria are commercially available, CDC can perform polymerase chain reaction (PCR) test on clinical specimens to confirm infection with a potentially toxigenic strain. PCR can detect nonviable *C. diphtheriae* organisms from specimens taken after antibiotic therapy has been initiated.

Although PCR for the diphtheria toxin gene[l] and its regulatory element, as performed by the CDC Pertussis and Diphtheria Laboratory, PCR and MALDI-TOF provide supportive evidence for the diagnosis but do not confirm toxin production. These tests, when used, should always be combined with a test that confirms toxin production, such as the Elek test.

**Isolation of *C. diphtheriae* by culture**
Isolation of *C. diphtheriae* by 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 site of diphtheria infection, nose, throat, and, if present, 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.

Specimens from the nose and throat (i.e., both a nasopharyngeal and a pharyngeal swab) for culture should be obtained from all patients with suspected diphtheria and their close contacts.

Isolation of *C. diphtheriae* from close contacts may confirm the diagnosis of the case, even if the patient's culture is negative.

**Biotype testing**
After *C. diphtheriae* has been isolated, the biotype (substrain) should be determined. The 4 biotypes are gravis, mitis, intermedius, and belfanti.

**Toxigenicity testing**
In addition to determining biotype, 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 testing**
Additional clinical specimens for PCR testing at CDC should be collected at the time specimens are 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 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). 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 Pertussis and Diphtheria Laboratory at 404-639-1231 or 404-639-1239.

**MALDI-TOF**
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-Tof) is an additional technology that can be used to rapidly identify bacterial species such as *C. diphtheriae*. The technique requires an isolate in order to identify the protein composition of microbial cells. However, this form of testing only confirms the bacterial species, does not confirm diphtheria toxin production, and is not available at the CDC Pertussis and Diphtheria Laboratory.

**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. High antibody levels may indicate protection against diphtheria, and *C. diphtheriae* infection is less likely to produce a serious illness. However, if antibody levels are low, diphtheria cannot be accurately ruled out. 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.

**Submission of *C. diphtheriae* isolates**
All isolates of *C. diphtheriae* from any anatomical site should be sent to the CDC Pertussis and Diphtheria Laboratory for reference testing. Clinical specimens from patients with suspected diphtheria for whom diphtheria antitoxin has been released for treatment should also be sent to the CDC Pertussis and Diphtheria Laboratory for culture, PCR, and toxigenicity testing. To arrange for shipping of specimens, contact your state health department.

## *B.* Haemophilus influenzae *type b (Hib)*

(see Chapter 2 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt02-hib.html])

**Presumptive identification by Gram stain or antigen detection**
The Gram stain is an empirical method for differentiating bacterial species into 2 large groups based on the chemical and physical properties of their cell walls. Gram-positive bacteria retain the primary stain while gram-negative bacteria take the color of the counterstain. A Gram stain can also serve to assess the quality of a clinical specimen. Gram stain is useful for preliminary identification of likely *H. influenzae*, though is not a confirmatory test and cannot distinguish among *H.influenzae* type b (Hib) serotypes.

Because the type b capsular antigen can be detected in body fluids, including urine, blood, and cerebrospinal fluid (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 or PCR, particularly in the diagnosis of patients who have received antimicrobial agents before specimens are obtained for culture. The method for antigen detection is latex agglutination, which is a rapid and sensitive method used to detect Hib capsular polysaccharide antigen in CSF; however, false-negative and false-positive reactions can occur.

If the Hib antigen is detected in CSF from a patient with meningitis but a positive result is not obtained from culture of sterile site, the patient should be considered as having 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.

**Confirmation by culture and PCR**
Confirming a case of Hib disease requires isolating *H. influenzae* or detecting *H. influenzae* DNA from a normally sterile body site. Normally, sterile sites for isolation of invasive *H. influenzae* typically include CSF, blood, joint fluid, pleural effusion, pericardial effusion, peritoneal fluid, subcutaneous tissue fluid, placenta, and amniotic fluid. Most hospital and commercial microbiologic laboratories have the ability to isolate *H. influenzae*. *H. influenzae* isolates are recommended to be tested for antimicrobial susceptibility to ampicillin, 1 of the third-generation cephalosporins, chloramphenicol, and meropenem. Further antimicrobial susceptibility testing should be considered for isolates obtained from cases in which a failure in treatment or chemoprophylaxis is suspected or in an outbreak setting.

Although culture is the gold standard for confirming *H. influenzae*, real-time PCR is an accepted alternative. In recent years, significant improvements have been made in both the sensitivity and specificity of PCR assays used for the detection of *H. influenzae*. Real-time PCR assays are available to detect DNA of H. influenzae and all 6 serotypes in blood, CSF, or other clinical specimens. A major advantage of PCR is that it allows for detection of *H. influenzae* from clinical samples in which the organism could not be detected by culture methods, such as when a patient has been treated with antibiotics before a clinical specimen is obtained for culture. Even when the organisms are nonviable following antimicrobial treatment, PCR can still detect *H. influenzae* DNA. Isolation of the bacterium is needed to test for antimicrobial susceptibility.

Several commercial multiplex PCR assays capable of simultaneously testing a single specimen for an array of pathogens that cause blood infections or meningitis/encephalitis are now available, primarily for clinical settings (e.g., FilmArray® Blood Culture Identification Panel and FilmArray® Meningitis/Encephalitis [ME] Panel from BioFire Diagnostics and Meningitis/Encephalitis Panel by PCR from ARUP Laboratories).[6–10] While such assays can rapidly identify *H. influenzae* (Hi) and *Neisseria meningitidis* (Nm) species, most do not determine serotype or serogroup. **Therefore, it is important for laboratories using assays that do not determine serotype/serogroup to perform either a simultaneous culture or a reflex culture if Hi or Nm is identified.** At a minimum, laboratories should collect and maintain an adequate clinical sample for further testing at a laboratory with a PCR assay that can detect serotype/serogroup.

**Serotype testing (serotyping)**
Serotyping distinguishes encapsulated strains, including Hib, from unencapsulated strains, which cannot be serotyped. The 6 encapsulated types (designated a–f) have distinct capsular polysaccharides that can be differentiated by slide agglutination with type-specific antisera.

To make public health decisions about chemoprophylaxis, microbiology laboratories should perform serotype testing of *H. influenzae* isolates and clinical specimens that are positive for *H. influenzae* in a timely manner.[11, 12] Even though Hib disease has declined, laboratories should continue routine serotyping. If serotyping is not available at a laboratory, laboratory personnel should contact the state health department. State health departments with questions about serotyping should contact the CDC Meningitis and Vaccine-Preventable Disease Branch laboratory at 404-639-3158.

**Molecular typing**
Although not widely available, whole genome sequencing (WGS) has been used to type the *H. influenzae* isolates to assess their genetic similarity. Subtyping the Hib bacterium by pulsed-field gel electrophoresis (PFGE),[13, 14] multilocus sequence typing (MLST), can also be performed for epidemiologic purposes in settings where WGS is not available. Some subtyping methods, such as outer membrane proteins, lipopolysaccharides, or enzyme electrophoresis, are no longer recommended or performed because they were unreliable or too labor intensive. The state health department may direct questions about subtyping to the CDC Meningitis and Vaccine-Preventable Disease Branch laboratory at 404-639-3158.

## C. Hepatitis A

(see Chapter 3 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt03-hepa.html])

Diagnostic tests used to confirm hepatitis A virus infection include serologic testing, and occasionally, 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 obtained as soon as possible after onset of symptoms because IgM anti-HAV generally disappears within 6 months after onset of symptoms.

Immunglobulin G (IgG) anti-HAV appears in the acute or 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 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 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt04-hepb.html])

Diagnostic tests used to confirm hepatitis B virus (HBV) infection include serologic testing, genotyping and subtyping (in outbreak investigations), and occasionally PCR-based assays to amplify/quantify and determine the sequence of viral genomes.

**Serologic testing**
Several well-defined antigen-antibody systems are associated with HBV infection, including the surface antigen of HBV (HBsAg) and antibodies to HBV surface antigen (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. 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 IgM class of anti-HBc, which persists for approximately 6 months. IgM anti-HBc may not be present in newly infected children younger than 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) of these persons may eventually clear HBsAg and develop anti-HBs.

In some cases, anti-HBc is the only serologic marker detected. Isolated anti-HBc can occur after HBV infection in persons who have recovered but whose anti-HBs levels have waned or in persons in whom anti-HBs failed to develop. Certain chronically infected persons may be positive for anti-HBc alone, with HBsAg levels that are below levels detectable by commercially available tests. Infants who are born to HBsAg-positive mothers and who do not become infected may also have detectable anti-HBc for up to 24 months after birth from passively transferred maternal antibody.

The diagnosis of acute hepatitis due to hepatitis B virus infection is serologically confirmed by a positive test for IgM antibody to hepatitis B core antigen (anti-HBc). If testing for IgM antiHBc is not available, the diagnosis of acute hepatitis B can also be confirmed by a positive test for HBsAg with a negative test for anti-HAV (Table 4). Confirmation of HBsAg-positive results by HBsAg neutralization assay should be done as needed according to the manufacturer's instructions in the package insert. In addition to acute HBV infection, both perinatal HBV infection and chronic HBV infection are reportable vaccine-preventable conditions. Chronic infection with HBV is confirmed by a positive test for HBsAg accompanied by a negative test for IgM anti-HBc or by 2 positive HBsAg test results that are at least 6 months apart. A diagnosis of perinatal HBV infection is confirmed by a positive test for HBsAg in an infant 1–24 months of age born in the United States or in U.S. territories to an HBsAg-positive mother.

**Table 4. Interpretation of hepatitis B serologic tests**

| Serologic Markers | | | | Interpretation |
|---|---|---|---|---|
| HBsAg* | Total Anti-HBc† | IgM Anti-HBc§ | Anti-HBs¶ | |
| - | - | - | - | Susceptible, never infected |
| + | - | - | - | Acute infection, early incubation** |
| + | + | + | - | Acute infection |
| - | + | + | - | Acute resolving infection |
| - | + | - | + | Past infection, recovered and immune |
| + | + | - | - | Chronic infection |
| - | + | - | - | False positive (i.e., susceptible), past infection, or "low level" chronic infection |
| - | - | - | + | Immune if titer is >10 mIU/ml |

* *Hepatitis B surface antigen.*
† *Antibody to hepatitis B core antigen.*
§ *Immunoglobulin M.*
¶ *Antibody to hepatitis B surface antigen.*
** *Transient HBsAg positivity (lasting <18 days) might be detected in some patients during vaccination.*

**Molecular analysis**
Molecular virologic methods such as PCR-based assays are available from CDC and commercial laboratories for detection and sequencing of HBV DNA. Although results for HBV DNA are not currently included in the definition for acute hepatitis B, they are included for the chronic HBV definition. Testing for HBV DNA is most commonly used for the purpose of evaluating a patient with diagnosed HBV infection who is receiving or being considered for treatment; these tests are not typically used for the initial diagnosis of infection.

PCR-based methods for amplifying and sequencing the HBV genome, done in conjunction with epidemiologic studies, may be helpful for investigating common-source outbreaks of hepatitis B infection. In addition, these assays are essential for detecting the emergence of vaccine-resistant strains. For example, detection of HBV variants or "escape mutants" among vaccinated infants of HBsAg-positive women is important to determine their potential role in vaccine failures.[15] Healthcare 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, 404-718-8500.

## *E. Influenza*

(updated as of October 2017; see Chapter 6 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt06-influenza.html])

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

**Virus isolation**
Virus isolation is the gold standard for influenza diagnosis. The following guidelines should be considered.

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 use immunologic methods to detect viral antigens in cell culture are available. These assays can provide results in 18–40 hours, compared with an average of 4–5 days to obtain positive results from standard culture.

**Molecular testing methods**
RT-PCR, including real-time RT-PCR, can be used to detect the presence of influenza virus in a clinical specimen or to characterize an influenza virus grown in tissue culture or embryonated eggs.

RT-PCR testing can be performed under biosafety level 2 conditions, even for viruses such as avian influenza A(H5N1), which require biosafety level 3 with enhancements for viral culture.

**Antigen detection assays**
Several methods exist for the diagnosis of 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 3 groups: the first detects only influenza type A viruses; 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.

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. The medium used to store the specimen for some rapid testing methods is inappropriate for viral culture; in this case, it is necessary to collect 2 separate samples. These additional or reserved samples may be used to confirm direct test results by culture and to subtype influenza A isolates.

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

Hemagglutination inhibition (HI) tests are the preferred method of serodiagnosis. A positive result is a 4-fold or greater rise in titer between the acute- and convalescent-phase samples when tested at the same time. Serologic test results are usually available in 24 hours.

Serologic testing is most useful in special studies; serologic diagnosis of influenza is not used for national surveillance because of the lack of standardized testing methods and interpretation.

## *F. Measles*

(see Chapter 7 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt07-measles.html])

**Serologic testing**
Serologic testing for antibodies to measles is widely available. Generally, in a susceptible person exposed to wild-type measles virus, the IgM response begins around the time of rash onset and can be detected for 1–2 months. The IgG response starts more slowly, at about 5–10 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 seroconvesion a 4-fold rise in the titer of IgG antibody in 2 serum specimens obtained approximately 2 weeks apart. Uninfected persons are IgM negative but will either be IgG negative or IgG positive, depending upon their previous disease or vaccination histories.

**Recommendations for serologic testing for measles**
An EIA test for IgM antibody to measles in a single serum specimen, obtained at the first contact with the suspected measles case-patient, is 1 of the recommended methods 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 seroconversion or a 4-fold 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 rash onset), neither IgM nor IgG antibody responses can distinguish measles disease from rash following vaccination. In this instance, a viral throat or nasopharyngeal swab specimen should be obtained so CDC can attempt to distinguish between vaccine virus and wild-type virus (Table 5).

**Table 5. 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 first 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 second dose of measles vaccine | Seroconversion. IgG response depends on timing of specimen collection. |
| - | + | Previously vaccinated, IgG+ | Recently received second 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. |
| + | + | 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.*

*Tests for IgM antibody.* Although multiple possible methods exist for testing for IgM antibody, EIA is the most consistently accurate test and is therefore the recommended method. There are 2 formats for IgM tests. The first and most widely available is the indirect format, which requires a specific step to remove IgG antibodies. Problems with removal of IgG antibodies can lead to false-positive[16] or, less commonly, false-negative results.

The second format, IgM capture, does not require the removal of IgG antibodies. This is the preferred reference test for measles.

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; 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.[17]

When a laboratory IgM test result is suspected of being false-positive, 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 yield false-positive reactions in some IgM tests for measles. False-positive tests may be suspected when thorough surveillance reveals no source or spread of cases, when the case does not meet the clinical case definition, or when the IgG result is positive within 3 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. Indirect EIA tests may be validated with capture EIA.

*Tests for IgG antibody.* Because tests for IgG require 2 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-phase) serum specimen, collected 14–30 days after the first (acute-phase) 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; these include EIA, 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-phase paired sera.

Paired IgG testing for laboratory confirmation of measles requires the demonstration of a 4-fold rise in titer of antibody against measles. The tests for IgG antibody should be conducted on both acute- and convalescent-phase 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 rises in titer.

**RT-PCR and Virus isolation**

Isolation of measles virus in culture or detection of measles virus by RT-PCR in clinical specimens confirms the diagnosis of measles. Since culture can take weeks to perform, RT-PCR is preferred for case confirmation. It is important to note that a negative culture or RT-PCR result does not rule out measles because the tests are greatly affected by the timing of specimen collection and the quality and handling of the clinical specimens. If measles virus is cultured or detected by RT-PCR, the viral genotype can be used for molecular epidemiology and to distinguish between measles disease caused by a wild-type measles virus and a measles vaccine reaction.

Viral culture and RT-PCR are important for molecular epidemiologic surveillance to help determine:

- the origin of the virus,
- which viral strains are circulating in the United States, and
- whether these viral strains have become endemic in the United States.

Specimens (urine, nasopharyngeal aspirates, heparinized blood, or throat swabs) from clinically suspected cases of measles obtained for virus isolation should be shipped to the state public health laboratory or to 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 7). Clinical specimens for virus isolation should be collected at the same time as samples 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 more than 10 days have passed after rash onset.

## *G.* Neisseria meningitiditis*, Meningococcal disease*

(see Chapter 8 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt08-mening.html])

**Identification of *N. meningitidis***

*Presumptive identification by Gram stain:* The Gram stain is an empirical method for differentiating bacterial species into 2 large groups based on the chemical and physical properties of their cell walls. Gram-positive bacteria retain the primary stain while gram-negative bacteria take the color of the counterstain. A Gram stain can also serve to assess the quality of a clinical specimen. Gram staining for *N. meningitidis* is commonly used and continues to be a reliable and rapid method for presumptive identification. Intracellular gram-negative diplococci in CSF can be considered meningococci until proven otherwise.

*Presumptive identification by antigen detection:* Latex agglutination can be used for rapid detection of meningococcal capsular polysaccharides in CSF; however, false-negative or false-positive results can occur. Antigen agglutination tests on serum or urine samples are unreliable for the diagnosis of meningococcal disease.[18] Immunohistochemistry can be used for meningococcal detection in formalin-fixed tissues.

Confirmation by culture and PCR: The case definition for confirmed meningococcal disease requires isolation of *N. meningitidis* or detection of Nm. DNA from a normally sterile site. Typically, normally sterile sites include blood or CSF, but can also include joint, pleural, or pericardial fluid. Aspirates or skin biopsies of purpura or petechiae can yield meningococci in cases of meningococcemia. The typical media used to grow the organism are chocolate agar or Mueller-Hinton medium in an atmosphere containing 5% carbon dioxide.[19]

Real-time PCR detects DNA of meningococci in blood, CSF, or other clinical specimens and is an acceptable alternative method for confirming meningococcal cases. Real-time PCR assays are available to detect DNA of *N. meningitidis* and all 6 serogroups in blood, CSF, or other clinical specimens.[6–10] A major advantage of PCR is that it allows for detection of *N. meningitidis* from clinical samples in which the organism could not be detected by culture methods, such as when a patient has been treated with antibiotics before a clinical specimen is obtained for culture. Even when the organisms are nonviable following antimicrobial treatment, PCR can still detect *N. meningitidis* DNA.[20] Because of the severity of meningococcal disease, it is critical to treat the patient as soon as infection is suspected and not delay to obtain a culture or laboratory results.

Several commercial multiplex PCR assays capable of simultaneously testing a single specimen for an array of pathogens that cause blood infections or meningitis/encephalitis are now available, primarily for clinical settings (e.g., FilmArray® Blood Culture Identification Panel and FilmArray® Meningitis/Encephalitis [ME] Panel from BioFire Diagnostics and Meningitis/Encephalitis Panel by PCR from ARUP Laboratories).[9,21–22] While such assays can rapidly identify Hi and Nm species, most do not determine serotype or serogroup. Therefore, it is important for laboratories using assays that do not determine serotype/serogroup to perform either a simultaneous culture or a reflex culture if Hi or Nm is identified. At a minimum, laboratories should collect and maintain an adequate clinical sample for further testing at a laboratory with a PCR assay that can detect serotype/serogroup.

**Serogroup testing**

Serogrouping distinguishes encapsulated meningococcal strains from unencapsulated strains, which do not express any capsule on cell surface and cannot be serogrouped. The 12 encapsulated groups (A, B, C, W, X, Y, etc.) have distinct capsular polysaccharides that can be differentiated by slide agglutination with specific antisera or PCR.

Microbiology laboratories should perform serogroup testing of meningococcal isolates and clinical specimens that are positive for *N. meningitidis* in a timely manner. If serogrouping is not available at a laboratory, laboratory personnel should contact the state health department. State health departments with questions about serogrouping should contact the CDC Meningitis and Vaccine Preventable Disease Branch laboratory at 404-639-3158.

**Susceptibility testing**

Routine antimicrobial susceptibility testing of meningococcal isolates is not recommended. *N. meningitidis* strains with decreased susceptibility to penicillin G have been identified sporadically from several regions of the United States, Europe, and Africa.[23] Most of these isolates with reduced penicillin susceptibility remain moderately susceptible (minimum penicillin inhibitory concentration of between 0.12 µg/mL and 1.0 µg/mL). High-dose penicillin G remains an effective treatment against moderately susceptible meningococci. Surveillance of susceptibility patterns in populations should be conducted to monitor trends in *N. meningitidis* susceptibility.

**Molecular typing during outbreaks**

Phenotypic and genotypic methods are used to investigate meningococcal diversity. Capsular polysaccharide (serogroup), porin protein PorB (serotype), and porin protein PorA (serosubtype) are all phenotypic characteristics that can be used to distinguish meningococci from one another.[24] Outbreaks of meningococcal disease are usually caused by the same or closely related strains.[25] Molecular genotyping techniques such as PFGE, 16S rRNA gene sequencing, or MLST are used for subtype characterization of an outbreak clone.[26,27] This subtyping helps to better define the extent of the outbreak. It is crucial to have rapid and reliable results in determining the meningococcal serogroup in an outbreak because public health response will differ for vaccine-preventable or nonvaccine–preventable disease. Molecular genotyping provides important tools for understanding the overall epidemiology of meningococcal disease, but

different methods may be more useful in certain settings. PFGE or 16S rRNA gene typing seem to be most useful for outbreak and short-time-period epidemiology, whereas MLST has become the "gold standard" for long-term, global tracing of meningococcal population changes.

## H. Mumps

(see Chapter 9 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt09-mumps.html])

Acute mumps infection can be confirmed by the detection of mumps virus RNA, by RT-PCR, or positive mumps virus isolation. The presence of serum mumps IgM or IgG seroconversion can also be used to aid in the diagnosis of mumps infection. A significant rise in IgG antibody in acute and convalescent-phase serum specimens may also be used for unvaccinated persons but is of limited value for previously vaccinated or previously infected persons because they generally present with high levels of IgG in the acute specimen.

Collection of both a viral specimen and a serologic specimen is recommended from all persons with clinical features compatible with mumps. Early collection (day 0 to 3) of a buccal or oral swab provides the best means of laboratory confirmation, particularly among persons with a history of vaccination. Virus may be isolated from the parotid gland/buccal mucosa from 6 days before until 10 days after salivary enlargement. Urine is less likely than oral specimens to contain sufficient virus for culture or detection; therefore, buccal swabs are preferred in persons with parotitis.[28] However, in patients presenting with orchitis, urine may be useful for diagnosis in addition to oral specimens.

Maximal viral shedding occurs 1–3 days prior to onset and through day 5 following onset of symptoms. Virus may be cleared more rapidly from vaccinated persons who become infected, so early collection of viral samples is critical. Among unvaccinated persons, IgM antibodies are detectable within 5 days after onset of symptoms, reach a maximum level about a week after onset of symptoms, and remain elevated for several weeks or months.[29,30] Previously vaccinated persons may not have an IgM response or the response may be transient or delayed. The likelihood of detecting mumps IgM is increased if serum is collected >3 days post parotitis onset. Prior immunization against mumps or previous natural infection may be documented by the presence of serum IgG mumps-specific antibodies by EIA. The level of IgG that provides immunity has not been established.

**Serologic testing for IgM antibody**

The serologic tests available for laboratory confirmation of mumps acute infections and measurement of mumps IgG vary greatly in sensitivity and specificity. The state health department can provide guidance on available laboratory services and preferred tests. Most IgM detection assays work well in unvaccinated persons.[31] Commercial IFA and indirect EIA tests are less sensitive than capture IgM assays.

*Enzyme immunoassay.* EIA is a highly specific test for diagnosing acute mumps infection. At the direction of the state health department, healthcare providers and state and local health departments may send serum specimens from persons with suspected mumps cases to the CDC Viral Vaccine-Preventable Diseases Branch for IgM detection by EIA.

*Immunofluorescence assays.* IFAs have the advantage of being relatively inexpensive and simple. The reading of IFA IgM tests requires considerable skill and experience since nonspecific staining may cause false-positive readings. IFA and indirect EIA may be susceptible to interference by high levels of IgG. Treatment of serum to remove IgM may be necessary to avoid false-positive results.

***Note:*** *Commercially available IFA antibody assays and EIA kits for detection of mumps IgM are currently not approved by the U.S. Food and Drug Administration (FDA). Each laboratory must validate these tests independently.*

**Virus isolation and RT-PCR**

Mumps virus can be isolated from fluid collected from the parotid duct (Stensen's duct) or other affected salivary gland ducts, and from the throat, CSF, and urine (although urine is less likely to yield virus and is therefore not recommended in those with parotitis). However, in patients presenting only with orchitis, urine may be useful for laboratory confirmation in addition to oral specimens. Parotid duct swabs yield the best sample, particularly when the salivary gland area is massaged approximately 30 seconds prior to collection of the buccal/parotid duct fluid. An effort should be made to obtain the specimen as soon as possible after parotitis or meningitis onset. Because few laboratories perform mumps virus isolation, 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 for mumps confirmation in appropriately collected specimens. Molecular epidemiologic surveillance makes it possible to build a sequence database that will help track transmission pathways of mumps strains circulating in the United States. In addition, typing methods are available to distinguish wild-type mumps virus from vaccine virus. Specimens for molecular typing should be obtained as soon as possible after the onset of parotitis, ideally from the day of onset to 3 days later (not more than 10 days after parotitis). Specific instructions for specimen collection and shipping may be obtained from CDC by contacting the Viral Vaccine-Preventable Diseases Branch. Specimens for virus isolation and molecular typing should be sent to CDC or the state health department.

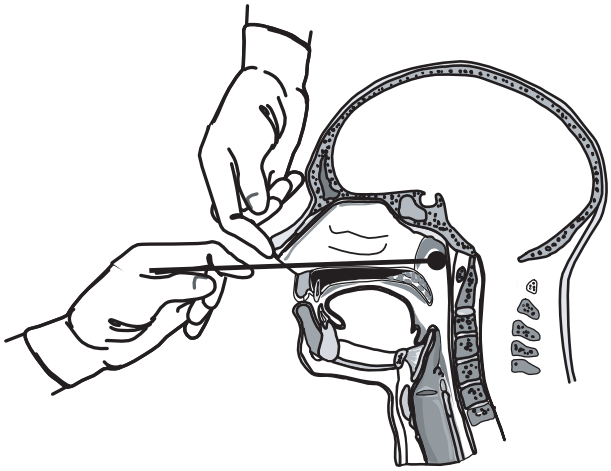
## *I. Pertussis*

(see Chapter 10 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt10-pertussis.html])

**Culture**
Isolation of *B. pertussis* by bacterial culture remains the gold standard for diagnosing pertussis. It is also required for antimicrobial susceptibility testing and molecular typing. 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.[32] The timing of specimen collection can affect the isolation rate, as can inadequately collected specimens and concurrent use of effective antimicrobial agents. Because patients can remain culture positive even while taking effective antibiotics (e.g., when strains 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 persons with suspected pertussis disease should have a nasopharyngeal aspirate or swab obtained from the posterior nasopharynx for culture. Recovery rates of *B. pertussis* from nasopharyngeal aspirates are similar to or higher than rates of recovery from nasopharyngeal swabs;[32–35] rates of recovery from throat and anterior nasal swabs are unacceptably low. Therefore, specimens should be obtained from the posterior nasopharynx, not the throat (Figure 1), by using a polyester (such as Dacron®) or nylon flocked nasopharyngeal swabs. Cotton-tipped swabs are not acceptable as residues present in these materials inhibit growth of *B. pertussis*. Specimens should be plated directly onto selective culture medium or placed in transport medium. Regan-Lowe agar or Bordet-Gengou agar are generally used for culture; half-strength Regan-Lowe should be used as the transport medium. Success in isolating the organism declines if the patient has received prior antibiotic therapy effective against susceptible *B. pertussis* (erythromycin or trimethoprim-sulfamethoxazole), if there is a delay in specimen collection beyond the first 2 weeks of illness, or if the patient has been vaccinated. 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.

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



**Polymerase chain reaction**

PCR testing of nasopharyngeal swabs or aspirates can be a rapid, sensitive, and specific method for diagnosing pertussis.[33,36] However, false-positive results may be obtained because of contamination in the laboratory or during specimen collection.[36,37] PCR is currently available in most laboratories; the assay varies among laboratories and is not standardized.[36] Direct comparison with culture is necessary for validation, and the use of multiple targets is recommended to distinguish *B. pertussis* from other *Bordetella* species.[38] Even if a laboratory has validated its PCR method, isolation of *B. pertussis* by culture should be attempted when possible. *B. pertussis* isolates can then be evaluated for azithromycin and erythromycin susceptibility and by molecular typing methods, which can help define the molecular epidemiology of strains circulating in the United States. Cotton-tipped or calcium alginate swabs are not acceptable to collect nasopharyngeal specimens as residues in these materials inhibit PCR assays.

**Serologic testing**

Although serologic testing has proved useful in clinical studies and outbreak investigations, it is not yet standardized. Also, the lack of association between antibody levels and immunity to pertussis makes results of serologic testing difficult to interpret. Despite the fact that serology is not part of the clinical case definition, commercial clinics and manufacturers still provide serology and serology kits for pertussis diagnosis. No serologic assay is FDA approved and very little is understood about the clinical accuracy of these commercially available serologic assays. Only in Massachusetts, where the state utilizes its own clinically validated assay, is serology used for clinical diagnosis and reporting for patients 11 years of age and older.[39] CDC, together with the FDA, validated an IgG anti-PT ELISA that has proven useful for diagnosis in adolescents and adults during the later phases of the disease.[40–42] Elsewhere, with few exceptions, it is not known 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 testing**

DFA testing of nasopharyngeal secretions is not recommended as a test for diagnosing 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 but the sensitivity and specificity are variable.

**Elevated white blood cell count**

An elevated white blood cell count 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$^3$ or higher. However, there may be no lymphocytosis in very young infants, vaccinated children, or adults with mild cases of pertussis. The white blood cell count is not a test of confirmation.

**Pulsed-field gel electrophoresis**
PFGE is a type of DNA fingerprinting. This technique has been a useful tool for distinguishing 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.[43,44]

**Multi-locus variable-number tandem repeat analysis**
Multi-locus variable-number tandem repeat analysis (MLVA) is a molecular typing method that compares genomic regions of direct repeats (variable-number tandem repeats, or VNTRs) between strains;[45] it demonstrates less diversity of strains than PFGE. MLVA has the advantage of being applicable to both cultures and directly to nucleic acid extracted from clinical specimens.[46]

**Multi-locus sequence typing**
MLST is another typing method that analyzes nucleotide variation in a predetermined set of genes or gene fragments.[47] MLST methods vary between bacterial species. Although an MLST method for *B. pertussis* is not standardized, many laboratories analyze fragments of 3 genes and promoter regions encoding virulence factors that are included in several acellular pertussis vaccines.[48,49]

Questions about performing PFGE on *B. pertussis* isolates, as well as questions about isolating *B. pertussis*, performing azithromycin or erythromycin susceptibility testing, and performing PCR can be directed to the Pertussis and Diphtheria Laboratory at CDC. Call Dr. M. Lucia Tondella at 404-639-1239, or Ms. Pam Cassiday at 404-639-1231. If needed, *B. pertussis* isolates can be sent to:

CDC, Pertussis and Diphtheria Laboratory
Attention: Pam Cassiday
DASH Unit 12
1600 Clifton Road NE
Atlanta, GA 30329

## *J. Pneumococcal infection*

(see Chapter 11 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt11-pneumo.html])

**Culture**
*Streptococcus pneumoniae* is a Gram-positive, lancet-shaped diplococcus that commonly inhabits the throat as normal flora. *S. pneumoniae* commonly causes lower and upper respiratory diseases, including pneumonia, meningitis, and acute otitis media. Diagnosis of invasive pneumococcal infection is confirmed by culture and isolation of *S. pneumoniae* from a normally sterile body site (e.g., blood, CSF, pleural fluid, peritoneal fluid). Alternatively, diagnosis can be confirmed from culture-negative specimens from normally sterile sites using real-time PCR.

**Antibiotic resistance**
The Clinical Laboratory Standards Institute (CLSI) recommends that clinical laboratories test all isolates of *S. pneumoniae* from CSF for resistance to penicillin, cefotaxime or ceftriaxone, meropenem, and vancomycin.[50] 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. Linezolid-resistance is extremely rare and has been associated with mutations within the rplD-encoded ribosomal protein L4.[51] For vancomycin, a strain is considered non-susceptible if it has a minimum inhibitory concentration of >1 µg/ml or greater or zone diameter less than <17 mm. For linezolid, nonsusceptible strains are those with a minimum inhibitory concentration (MIC) of >2 µg/ml or zone diameter <21 mm. Strains found to be nonsusceptible to vancomycin or linezolid 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 CLSI document for testing recommendations.[50] The CDC's Antibiotic Resistance Laboratory Network (ARLN) is available to assist state public health laboratories with susceptibility testing of IPD isolates. States can request testing for select IPD isolates. Contact ARLN@cdc.gov for information on this program. Additionally, isolates with unusual resistance features can be sent to the CDC Streptococcus Laboratory for phenotypic verification and genomic analysis employing their specialized bioinformatics pipeline for detecting resistance

determinants.[52] Contact pneumococcus@cdc.gov for additional information. State health department laboratories that obtain penicillin binding protein gene sequencing information may consider using the CDC Streptococcus Laboratory database for deducing β-lactam antibiotic MICs (https://www.cdc.gov/streplab/mic-tables.html).[53]

**Serotyping**

Current pneumococcal vaccines are based upon capsular polysaccharides. There are currently >91 known capsular serotypes. Since only subsets of capsular serotypes are included in pneumococcal vaccines, serotyping allows the measurement of vaccine efficacy and can provide data for development of expanded-serotype vaccines.[54] CDC and its partners perform active, population-based surveillance for invasive pneumococcal serotypes in specific areas that represent about 30 million people in the United States. Serotyping is currently performed in only a limited number of state public health laboratories, academic centers, or at CDC. State public health laboratories may consider adopting a PCR-based technique for determining capsular serotypes, http://www.cdc.gov/vaccines/pubs/surv-manual/chpt22-lab-support.html#f40.[55–58] The CDC Streptococcus Laboratory provides numerous protocols and references for state health departments and clinical labs to identify pneumococcal serotypes using PCR (https://www.cdc.gov/streplab/pcr.html). If states are unable to perform PCR serotyping, the CDC's ARLN and VPD programs can provide serotyping assistance for select IPD isolates or specimens. Contact ARLN@cdc.gov for serotyping assistance. The CDC Streptococcus Laboratory will conduct serotyping of pneumococcal isolates from blood, CSF, or other sterile sites in outbreak settings, and when appropriate will perform whole genome sequence analysis to determine strain features and relatedness between isolates from disease clusters. Contact pneumococcus@cdc.gov for outbreak assistance. Serotypes are deduced through the same bioinformatics pipeline used to determine MLST types and resistance features.[59]

## *K. Poliomyelitis*

(see Chapter 12 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt12-polio.html])

**Virus isolation**

The likelihood of poliovirus isolation is highest from stool specimens, intermediate from pharyngeal swabs, and very low from blood or spinal fluid. Poliovirus is present in the stool in the highest concentration and for the longest time of any specimen, and therefore remains the most critical specimen for diagnosis. Because cell culture is extremely sensitive for the detection of poliovirus, it remains as sensitive, or more sensitive, than most molecular assays. A negative pan-enterovirus PCR result cannot rule out poliovirus infection. Isolation of poliovirus from stool specimens contributes to the diagnostic evaluation but does not constitute proof of a causal association between the isolated viruses and paralytic poliomyelitis.[56] Isolation of virus from CSF is diagnostic but is rarely accomplished. To increase the probability of poliovirus isolation, at least 2 stool specimens and 2 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 14 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. CDC can assist with culture if it is not otherwise readily available.

Isolation of wild poliovirus constitutes a public health emergency, and appropriate control efforts must be immediately initiated (in consultation among healthcare providers, the state and local health departments, and CDC). Type 2 poliovirus has been declared eradicated; type 2 infectious and potentially infectious materials should be handled only in a proper containment facility.

**Serologic testing**

Serology may be helpful in supporting or ruling out the diagnosis of paralytic poliomyelitis. An acute-phase serum specimen should be obtained as early in the course of disease as possible, and a convalescent-phase specimen should be obtained at least 3 weeks later. A 4-fold rise in titer between the acute- and convalescent-phase specimens suggests poliovirus infection. Nondetectable 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, so that a 4-fold rise may not be

demonstrated. Vaccinated persons would also be expected to have measurable titers; therefore, vaccination history is important for interpretation of serologic tests. 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.

## *L. Rotavirus*

(see Chapter 13 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt13-rotavirus.html])

Laboratory testing is necessary to confirm group A rotavirus infection and to ensure reliable surveillance and clinical therapy. Because rotavirus is shed in such high concentrations in stool, fecal specimens are preferred for diagnosis of rotavirus. Methods available to diagnose rotavirus infection include detection of viral antigens (EIA, immunochromatography, electron microscopy, and immunostaining) and molecular detection by RT-PCR/quantitative reverse transcription PCR (qRT-PCR) and genomic sequencing.[57–59]

**Detection of viral antigens**
The most widely available method of antigen detection in stool is EIA, which detects an antigen common to all group A rotaviruses.[57] Several inexpensive commercial EIA kits are available and provide rapid and highly sensitive results (90%–100%). Because EIA is rapid, inexpensive, and highly sensitive, it is the most appropriate method for clinical diagnosis and surveillance.

Another less frequently used method more appropriate for a research setting is visualization of viral particles by electron microscopy.

**Molecular detection**
Molecular methods can be used to detect rotavirus infection in clinical specimens and to characterize the virus. Molecular methods for detection of viral RNA include RT-PCR, qRT-PCR, and genomic sequencing.[58,59]

- Multiplexed, semi-nested RT-PCR genotyping and genomic sequencing are widely used to identify the most common and several uncommon rotavirus G and P genotypes.[58,59]
- Genomic sequencing has been used extensively to identify uncommon strains and genetic variants that cannot be identified by RT-PCR genotyping and to confirm the results of genotyping methods.[58,59]

**Virus isolation**
Rotavirus can be isolated directly from fecal specimens by inoculation of cell cultures in the presence of trypsin-containing growth medium, but this procedure is labor-intensive and more appropriate for research laboratories.

## *M. Rubella*

(see Chapter 14 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt14-rubella.html])

Clinical diagnosis of rubella is unreliable, therefore, cases must be laboratory confirmed. Virus detection and serologic testing can be used to confirm acute or recent rubella infection. Serologic tests can also be used to screen for rubella immunity.

**Virus detection (real-time RT-PCR, RT-PCR)**
Rubella virus can be detected from nasal, throat, urine, blood, and CSF specimens from persons with rubella (see Appendix 15 at https://www.cdc.gov/vaccines/pubs/surv-manual/appx/appendix15-rubella.pdf). The best results come from throat swabs. CSF specimens should be reserved for persons with suspected rubella encephalitis. Efforts should be made to obtain clinical specimens for virus detection from all case-patients at the time of the initial investigation. Virus may be detected from 1 week before to 2 weeks after rash onset. However, maximum viral shedding occurs up to day 4 after rash onset.

Real-time RT-PCR and RT-PCR can be used to detect rubella virus and has been extensively evaluated for its usefulness in detecting rubella virus in clinical specimens.[60,61] Clinical specimens obtained for virus detection and sent to CDC are routinely screened by these techniques.

Molecular typing is recommended because it provides important epidemiologic information to track the epidemiology of rubella in the United States now that rubella virus no longer continuously circulates in

this country. By comparing virus sequences obtained from new case-patients with other virus sequences, the origin of particular virus types in this country can be tracked.[62] Furthermore, this information may help in documenting the maintenance of the elimination of endemic transmission. In addition, genotyping methods are available to distinguish wild-type rubella virus from vaccine virus.

**Serologic testing**

The serologic tests available for laboratory confirmation of rubella infections and immunity vary among laboratories. The state health department can provide guidance on available laboratory services and preferred tests. EIAs are the most commonly used and widely available diagnostic test for rubella IgG and IgM antibodies and are sensitive and relatively easy to perform. EIA is the preferred testing method for IgM, using the capture technique, although indirect assays are also acceptable.

Latex agglutination tests appear to be sensitive and specific for screening when performed by experienced laboratory personnel. Other tests in limited use to detect rubella-specific IgM include HI and IFA

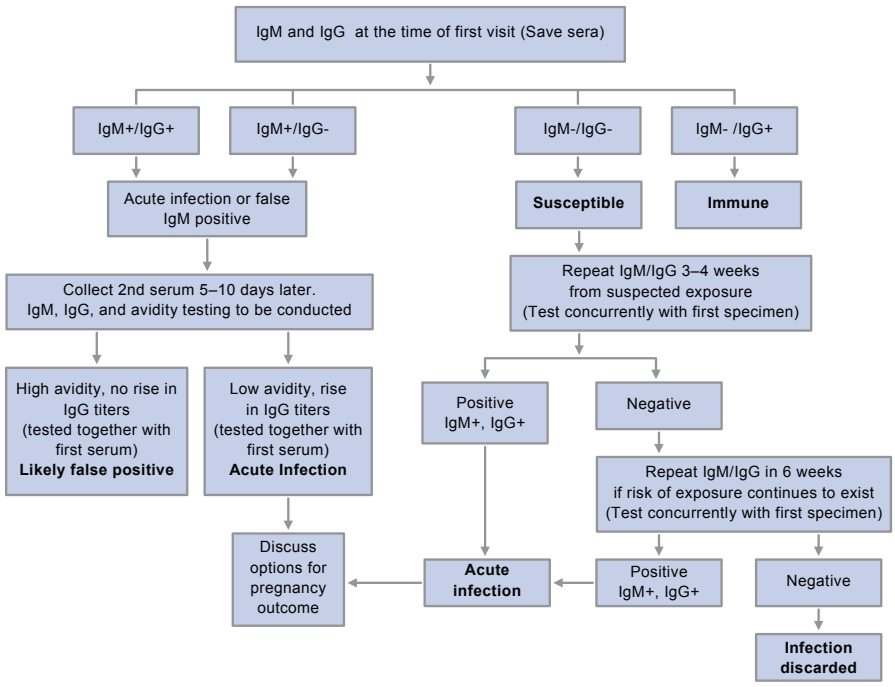
**Detection of IgM antibody**

Rubella-specific IgM can usually be detected 4–30 days after onset of illness, and often for longer. Sera should be collected as early as possible after onset of illness. However, IgM antibodies may not be detectable before day 5 after rash onset. In case of a rubella IgM-negative result in specimens taken before day 5, serologic testing should be repeated on a specimen collected after day 5.

Because rubella incidence is low, a high proportion of IgM-positive tests will likely be false positive. False-positive serum rubella IgM tests may occur due to the presence of rheumatoid factors (indicating rheumatologic disease) or cross-reacting IgM, or infection with other viruses.[63,64] Avidity testing (see below) and detection of wild-type rubella virus can be used to resolve uncertainties in the serologic evaluation of suspected cases.

Particular care should be taken when rubella IgM is detected in a pregnant woman with no history of illness or contact with a rubella-like illness. Although this is not recommended, many pregnant women with no known exposure to rubella are tested for rubella IgM as part of their prenatal care. If rubella test results are IgM-positive for persons who have no or low risk of exposure to rubella, additional laboratory evaluation should be conducted. Laboratory evaluation is similar to that described in the IgM-positive section of Figure 1.

**Figure 1. Algorithm for serologic evaluation of pregnant women exposed to rubella**



**Detection of IgG antibody (significant rise or avidity) for diagnostic testing**
To detect a significant rise in rubella-specific IgG concentration, the first serum specimen should be obtained as soon as possible after onset of illness and the second serum sample should be collected about 7–21 days after the first specimen. In most rubella cases, rubella IgG is detectable by 8 days after rash onset.[65] Tests for IgG antibody should be conducted on both acute-and convalescent-phase specimens at the same time with the same test.

Assays for IgG avidity are useful to distinguish the difference between recent and past rubella infections. Low avidity is associated with recent primary rubella infection, whereas high avidity is associated with past infection or reinfection. Avidity tests are not routine tests and should be performed in reference laboratories. A number of avidity assays have been described.[66, 67]

**Detection of IgG antibody to screen for rubella immunity**
A single serologic IgG test may be used to determine the rubella immune status of persons whose history of rubella disease or vaccination is unknown. The presence of serum IgG rubella-specific antibodies indicates immunity to rubella.

## *N. Congenital rubella syndrome*

(see Chapter 15 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt15-crs.html])

**Virus detection (real-time RT-PCR, RT-PCR)**
Rubella virus can be detected from nasal, throat, urine, and blood specimens from infants with CRS. Efforts should be made to obtain clinical specimens for virus isolation from infants at the time of the initial investigation (see Appendix 15, https://www.cdc.gov/vaccines/pubs/surv-manual/appx/appendix15-rubella.pdf). However, because infants with CRS may shed virus from the throat and urine for a prolonged period (a year or longer), specimens obtained later may also yield rubella virus. As with rubella infection, molecular typing is recommended because it provides important epidemiologic information to track the epidemiology of rubella in the United States now that rubella virus no longer continuously circulates in this country. By comparing virus sequences from new case-patients with virus sequences from other cases, the origin of particular virus types in this country can be tracked.[68] Furthermore, this information may help in documenting the maintenance of the elimination of endemic rubella virus transmission. Specimens for molecular typing should be obtained from patients with CRS as soon as possible after diagnosis. Appropriate specimens include throat swabs, urine, and cataracts from surgery. Specimens for virus detection and molecular typing should be sent to CDC as directed by the state health department.

## *Serologic testing*

The serologic tests available for laboratory confirmation of CRS infections vary among laboratories. EIAs are the most commonly used and widely available diagnostic test for rubella IgG and IgM antibodies. EIAs are sensitive and relatively easy to perform. EIA is the preferred testing method for IgM, using the capture technique, although indirect assays are also acceptable. In infants with CRS, IgM antibody can be detected in the infant's cord blood or serum and persists for about 6–12 months.

## *O. Varicella*

(see Chapter 17 [http://www.cdc.gov/vaccines/pubs/surv-manual/chpt17-varicella.html])

Laboratory confirmation of varicella has become more important due to the common vaccine-modified presentation of the disease, and more limited experience by physicians with unmodified disease. In addition, vaccine wildtype discrimination testing is important for the confirmation of vaccine-associated disease. Because varicella is the most common disease confused with smallpox, rapid laboratory confirmation of varicella zoster virus (VZV) diagnosis is required in cases of vesicular/pustular rash illness that fall into the category of "moderate risk" for smallpox according to the CDC algorithm. Diagnostic tests used to confirm recent varicella infection include detection and characterization of viral DNA.

**Rapid varicella zoster virus identification**
Realtime PCR techniques are the gold standard for confirming VZV infection. Viral DNA may also be detected in CSF in cases of neurologic disease and vasculopathies associated with VZV infection. If PCR results on CSF are negative, a VZV IgG intrathecal antibody assay may yield a positive result

as this method may be used to implicate VZV as much as one month after disease onset. Viral DNA is less frequently detected if more than 10 days post-onset of symptoms. Suitable samples for PCR testing include vesicular swabs, scabs from crusted lesions, saliva, CSF, and biopsy or autopsy samples from cases of suspected disseminated VZV disease. Other direct detection methods such as DFA are generally not recommended due to limitations in both sensitivity and specificity.

PCR is a powerful technique that permits the rapid amplification of specific sequences of viral DNA that would otherwise be present in clinical specimens at concentrations well below detectable limits. Carefully designed 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. Techniques have been developed that can distinguish vaccine markers from wild type in real time, making it possible to complete a test in a single day.

Postvaccination situations for which specimens should be tested include: 1) rash with more than 50 lesions occurring 7 or more 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, 404-639-2192, or email dds1@cdc.gov or vzvlab@cdc.gov for details about collection and submission of specimens for testing. The Vaccine Preventable Disease Reference Centers located in the state laboratories of California, Wisconsin, New York, and Minnesota are also capable of discriminating varicella vaccine from wild type VZV.

**Virus culture**
Virus culture for VZV is no longer recommended because of the time required to obtain a result and the relative insensitivity of this approach compared with PCR.

**Serologic testing**
Serologic tests are available for IgG (acute and convalescent) and IgM antibodies to VZV for confirmation of disease. 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. VZV IgM can confirm recent active VZV infection but may not always confirm primary infection. In addition, IgM methods may be prone to producing false positive results. Although not widely available, VZV IgG avidity can distinguish between primary VZV infection and past infection (reactivation of VZV will drive the transient expression of IgM, just as in a primary response. The National VZV Laboratory at CDC has developed a reliable IgM capture assay. Call 404-639-0066, 404-639-2192, or email dds1@cdc.gov or kjr7@cdc.gov for details about collection and submission of specimens for testing.

## References

1. Mothershed EA, et al. Development of a real-time PCR assay for rapid detection of the diphtheria toxin gene. *J Clin Microbiol* 2002;40:4713-9.
2. CDC. Centers for Disease Control and Prevention. Measles lab manual from the World Health Organization (Isolation and identification of measles virus in cell culture) [updated 2014NOV3; accessed 20175SEP 21]. http://www.cdc.gov/measles/lab-tools/WHO-lab-manual.html
3. CDC. Division of Scientific Resources (DSR) [updated 2011 APR 8; accessed 2012 OCT 10]. https://www.cdc.gov/ncezid/dsr/specimen-management-branch.html
4. CDC. Surveillance Resource Center:Methods https://www.cdc.gov/surveillancepractice/guide.html
5. Mothershed EA, Cassiday PK, Pierson K, Mayer LW, Popovic T, et al. Development of a real-time PCR assay for rapid detection of the diphtheria toxin gene. *J Clin Microbiol* 2002;40:4713-9.
6. Wang X, Mair R, Hatcher C, et al. Detection of bacterial pathogens in Mongolia meningitis surveillance with a new real-time PCR assay to detect *Haemophilus influenzae*. IJMM 2011. 301: 303–309.
7. Vuong J, Collard JM, Whaley MJ, et al. Development of real-time PCR methods for the detection of bacterial meningitis pathogens without DNA extraction. *PLoS One* 2016;11(2):e0147765.

8. Wang X, MJ Theodore, R Mair, et al. Clinical validation of multiplex real-time PCR assays for detection of bacterial meningitis pathogens. *J Clin Microbiol* 2012. 50702–708.
9. Leber AL, Everhart K, Balada-Llasat J-M, et al. Multicenter evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol* 2016. 54:2251–2261. doi:10.1128/JCM.00730-16
10. Altun O, Almuhayawi M, Ullberg M, Özenci V. Rapid identification of microorganisms from sterile body fluids by use of FilmArray. *J Clin Microbiol* 2015;53(2):710–2. doi: 10.1128/JCM.03434-14
11. CSTE.Vaccine preventable disease surveillance and reporting. CSTE position statement ID-9 1999. Atlanta, GA: CSTE; 2000.
12. CDC. Serotyping discrepancies in *Haemophilus influenzae* type b disease—United States, 1998–1999. *MMWR* 2002;51(32):706–7.
13. Fry AM, Lurie P, Gidley M, et al. *Haemophilus influenzae* type b disease among Amish children in Pennsylvania: reasons for persistent disease. *Pediatrics* 2001;108(4):E60.
14. Lucher LA, Reeves M, Hennessey T, et al. Reemergence, in southwestern Alaska, of invasive *Haemophilus influenzae* type b disease due to strains indistinguishable from those isolated from vaccinated children. *J Infect Dis* 2002;186:958–65.
15. Thomas HC, Carman WF. Envelope and precore/core variants of hepatitis B virus. *Gastroenterol Clin North Am* 1994;23:499–514.
16. Jenkerson SA, Beller M, Middaugh JP, Erdman DD. False-positive rubeola IgM tests. *N Engl J Med* 1995;332:1103–1104.
17. Hefland F, Heath JL, Anderson LJ, Maes EF, Guris D, Bellini WJ. Diagnosis of measles with an IgM capture EIA: the optimal timing of specimen collection after rash onset. *J Infect Dis* 1997;175:195–9.
18. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. *N Engl J Med* 2001;344:1378–88.
19. Granoff DM, et al. Meningococcal vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines 4th ed. Philadelphia, PA: Saunders; 2003:959–87.
20. Mothershed EA, Sacchi CT, Whitney AM, et al. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. *J Clin Microbiol* 2004;42:320–8.
21. Biofire FilmArray® Panels. Available at: http://www.biofiredx.com/products/the-filmarray-panels
22. ARUP Meningitis/Encephalitis Panel by PCR. Available at: http://ltd.aruplab.com/Tests/Pub/2013305
23. American Academy of Pediatrics. 2006 Meningococcal infections. In: Pickering LK, et al, editors. Red book: 2006 Report of the Committee on Infectious Diseases. Elk Grove Village, IL: American Academy of Pediatrics; 2006. p.452–60.
24. Raghunathan PL, Bernhardt SA, Rosenstein NE. Opportunities for control of meningococcal disease in the United States. Annu Rev Med 2004;55:333–53.
25. CDC. Prevention and control of meningococcal disease. *MMWR ecomm Rep* 2005;54(No. RR-7):1–17.
26. Sacchi CT, Whitney AM, Reeves MW, Mayer LW, Popovic T. Sequence diversity of *Neisseria meningitidis* 16S rRNA genes and use of 16S rRNA gene sequencing as a molecular subtyping tool. *J Clin Microbiol*
27. Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci* USA 1998;95:3140–5.
28. Tolpin MD, Schauf V. Mumps virus 1984. Belsche RA, editor. Textbook of human virology. Littleton, MA: PSG Publishing Company, p. 311–32.
29. Ukkonen P, Granström ML, Penttinen K. Mumps-specific immunoglobulin M and G antibodies in natural mumps infection as measured by enzyme-linked immunosorbent assay. *J Med Virol* 1981;8:131–42.
30. Benito RJ, Larrad L, Lasierra MP, Benito JF, Erdociaín F. Persistence of specific IgM antibodies after natural mumps infection. *J Infect Dis* 1987;155:156–7.

31. Rota JS, Rosen JB, Doll MK, et al. Comparison of the sensitivity of laboratory diagnostic methods from a well-characterized outbreak of mumps in New York City, 2009. *Clin Vaccine Immunol* 2013; 20(3):391–6. doi: 10.1128/CVI.00660-12
32. Bejuk D, Begovac J, Bace A, et al. Culture of *Bordetella pertussis* from three upper respiratory tract specimens. *Pediatr Infect Dis J* 1995;14:64–5.
33. Halperin SA, Bortolussi R, Wort AJ. Evaluation of culture, immunofluorescence, and serology for the diagnosis of pertussis. *J Clin Microbiol* 1989;27:752–7.
34. Hallander HO, Reizenstein E, Renemar B, Rasmuson G, Mardin L, Olin P. Comparison of nasopharyngeal aspirates with swabs for culture of *Bordetella pertussis*. *J Clin Microbiol* 1993;31:50–2.
35. Hoppe JE, Weiss A. Recovery of *Bordetella pertussis* from four kinds of swabs. *Eur J Clin Microbiol* 1987;6:203–5.
36. Meade BD, Bollen A. Recommendations for use of the polymerase chain reaction in the diagnosis of *Bordetella pertussis* infections. *J Med Microbiol* 1994;41:51–5.
37. Mandal S, Tatti KM, Woods-Stout D, et al. Pertussis pseudo-outbreak linked to specimens contaminated by *Bordetella pertussis* DNA from clinic surfaces. *Pediatrics* 2012;129:e424–30.
38. Tatti KM, Sparks KN, Boney KO, Tondella ML. Novel multitarget real-time PCR assay for rapid detection of *Bordetella* species in clinical specimens. *J Clin Microbiol* 2011;49:4059–66.
39. Marchant CD, Loughlin AM, Lett SM, et al. Pertussis in Massachusetts, 1981–1991: incidence, serologic diagnosis, and vaccine effectiveness. *J Infect Dis* 1994;169:1297–1305.
40. Menzies SL, Kadwad V, Pawloski LC, Lin T, Baughman AL, Martin M, Tondella M-LC, Meade BD, and the Pertussis Assay Working Group. Development and analytical validation of an immunoassay for quantifying serum anti-pertussis toxin antibodies resulting from *Bordetella pertussis* infection. Clinical and Vaccine Immunology 2009;16:1781–88.
41. Baughman AL, Bisgard KM, Edwards KM, et al. Establishment of diagnostic cutoff points for levels of serum antibodies to pertussis toxin, filamentous hemagglutinin, and fimbriae in adolescents and adults in the United States. *Clin Diagn Lab Immunol*. 2004;11(6):1045–53.
42. Burgos-Rivera B, Lee AD, Bowden KE, et al. Evaluation of level of agreement in *Bordetella* Species identification in three U.S. laboratories during a period of increased pertussis. *J Clin Microbiol* 2015;53:1842–4.
43. Schmidtke AJ, Boney KO, Martin SW, Skoff TH, Tondella ML, Tatti KM. Population diversity among *Bordetella pertussis* isolates, United States, 1935–2009. *Emerging Infectious Diseases* 2012;18:1248–55.
44. de Moissac YR, Ronald SL, Peppler MS. Use of pulsed-field gel electrophoresis for epidemiological study of *Bordetella pertussis* in a whooping cough outbreak. *J Clin Microbiol* 1994;32:398–402.
45. Bisgard K, Christie C, Reising S, et al. Molecular epidemiology of *Bordetella pertussis* by pulsed-field gel electrophoresis profile: Cincinnati, 1989–1996. *J Infect Dis* 2001;183:1360–67.
46. Schouls LM, van der Heide HG, Vauterin L, Vauterin P, Mooi FR. Multiple-locus variable-number tandem repeat analysis of Dutch *Bordetella pertussis* strains reveals rapid genetic changes with clonal expansion during the late 1990s. *J Bacteriol* 2004;186:5496–5505.
47. Bowden KE, Williams MM, Cassiday PK, et al. Molecular epidemiology of the pertussis epidemic in Washington State in 2012. *J Clin Microbiol* 2014;52:3549–57.
48. Litt DJ, Jauneikaite E, Tchipeva D, Harrison TG, Fry NK. Direct molecular typing of *Bordetella pertussis* from clinical specimens submitted for diagnostic quantitative (real-time) PCR. *J Med Microbiol* 2012;61:1662–68.
49. Maiden MC. Multilocus sequence typing of bacteria. *Annual Review of Microbiology* 2006;60: 561–88.
50. Clinical and Laboratory Standards Institute. M100-S22 Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement. Approved Standard. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.

51. Dong W, Chochua S, McGee L, Jackson D, Klugman KP, Vidal JE. Mutations within the rplD gene of linezolid-nonsusceptible *Streptococcus pneumoniae* strains isolated in the United States. *Antimicrob Agents Chemother* 2014;58(4):2459–62. doi: 10.1128/AAC.02630-13.
52. Metcalf BJ, Chochua S, Gertz RE, Jr., Li Z, Walker H, Tran T, et al. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive pneumococcal disease isolates recovered in the United States. *Clin Microbiol Infect* 2016;22(12):1002 e1–e8.
53. Li Y, Metcalf BJ, Chochua S, Li Z, Gertz RE, Jr., Walker H, et al. Penicillin-binding protein transpeptidase signatures for tracking and predicting beta-lactam esistance levels in *Streptococcus pneumoniae*. *MBio* 2016;7(3).
54. Beall B, McEllistren MC,,Gertz RE Jr, et al. Pre- and post-vaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. Active Bacterial Core Surveillance Team. *J Clin Microbiol* 2006;44:999–1017.
55. Pai R, Gertz RE, Beall B, et al. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. *J Clin Microbiol* 2006;44(1):124–31. doi: 10.1128/JCM.44.1.124-131.2006
56. Carvalho MG, Tondella ML, McCaustland K, et al. Evaluation and Improvement of real-time PCR detection assays to lytA, ply, and psaA genes for detection of pneumococcal DNA. *J Clin Microbiol* 2007:45(8);2460–66. doi: 10.1128/JCM.02498-06
57. Dias CA, Teixeira LM, Carvalho MG, Beall B. et al. Sequential multiplex PCR for determining capsular serotypes of pneumococci recovered from Brazilian children. *J Med Microbiol* 2007;56(9):1185–8. DOI: 10.1099/jmm.0.47347-0
58. Morais M, Carvalho MG, Roca, A, et al. Sequential multiplex PCR for identifying pneumococcal capsular serotypes from south-Saharan African clinical isolates. *J Med Microbiol* 2007;56:1181–4. doi: 10.1099/jmm.0.47346-0
59. Strebel PM, Sutter RW, Cochi SL, et al. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. *Clin Infect Dis* 1992;14:568–79. https://doi.org/10.1093/clinids/14.2.568
60. Gray JD, Desselberger U, editors. 2000. Rotavirus, methods & protocols. (Methods in molecular medicine). New York: Humana Press, 2000.
61. Fischer TK, Gentsch JR. Rotavirus typing methods and algorithms. *Rev Med Virol* 2004;14:71–82.
62. Esona MD, Gautam R. Rotavirus. Diagnostic Testing for Enteric Pathogens. (Clinics in Laboratory Medicine). Philadelphia: Elsevier, 2015.
63. Namuwulya P, Abernathy E, Bukenya H, et al. Phylogenetic analysis of rubella viruses identified in Uganda, 2003–2012. *J Med Virol* 2014; 86(12):2107–13.
64. Abernathy E, Cabezas C, Sun H, et al. Confirmation of rubella within 4 days of rash onset: comparison of rubella virus RNA detection in oral fluid with immunoglobulin M detection in serum or oral fluid. *J Clin Microbiol* 2009;47(1):182–88.
65. Lazar M, Abernathy E, Chen M-H, Icenogle J, et al. Epidemiological and molecular investigation of a rubella outbreak, Romania, 2011 to 2012. *Euro Surveill* 2016;21(38):30345.
66. Kurtz JB, Anderson MJ. Cross-reactions in rubella and parvovirus specific IgM tests. *Lancet* 1985;2:1356.
67. Morgan-Capner P. False positive tests for rubella-specific IgM. *Pediatr Infect Dis J* 1991;10:415–6.
68. Bosma TJ, et al. PCR for detection of rubella virus RNA in clinical samples. *J Clin Microbiol* 1995;33:1075–9.