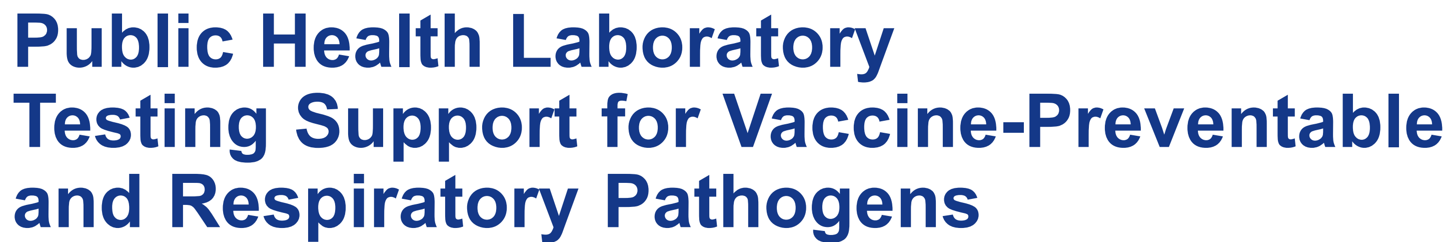


Public Health Laboratory Testing Support for Vaccine-Preventable and Respiratory Pathogens

June 2009

Association of Public Health Laboratories

Executive Summary

Background

In August and September 2007, the Association of Public Health Laboratories surveyed state public health laboratories to assess the volume of testing and current methodologies used for a variety of vaccine-preventable (VPDs) and respiratory disease pathogens and to establish benchmarks for future comparison. This report summarizes data from the 60 state and local public health laboratories that completed the survey.

Methods

APHL member laboratories, including those in states, local areas, territories and the District of Columbia, were invited via an explanatory email to participate in this pre-tested, self-administered, web-based, 41-question survey. Results were exported into SAS® (Statistical Analysis Software) Version 9.1 for subsequent analysis. Survey questions asked about testing for the following bacterial pathogens: *Bordetella* spp., *Clostridium tetani, Corynebacterium diphtheriae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia psittaci, Legionella* spp., *Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae* and Group A *Streptococcus*. Viral pathogens included measles, mumps, rubella and varicella.

Results

Of the 95 surveys sent out, 68 responses were received, representing a response rate of 72%. Of these, 46 state public health laboratories (SPHLs) and 14 local public health laboratories (LPHLs) finished the laboratory testing-based survey. Results are reported for "any" pathogens (greater than or equal to 1) and "all" pathogens (all of the listed) for the following testing categories: 1) cultures performed on-site and sent out; 2) polymerase chain reaction (PCR), direct fluorescent antibody (DFA) and serotyping/serogrouping performed on-site and sent out; and 3) serologic testing performed on-site. Data received from respondents with complete survey responses are displayed in **Tables 1 and 2**, "Public Health Laboratory Bacterial Testing Compacity" and "Public Health Laboratory Viral Testing Capacity." Pathogen-specific data are presented in Appendices A–I in the more inclusive document following this executive summary.

Discussion

Over the past three decades, many new laboratory testing technologies have been developed; however, culture is considered the gold standard and remains the most specific of the laboratory tests for many of the bacterial and viral pathogens. Almost all (93%) of the SPHLs and over one-half (57%) of the LPHLs surveyed provide on-site culture for any of the bacterial agents listed in **Table 1**. Many (65%) SPHLs and one-half (50%) LPHLs sent out specimens for additional testing — most often for definitive identification or confirmation or sub-typing. Viral isolation, although not always the test of choice for clinical diagnosis for the viruses discussed in this survey, is an important tool for epidemiologic viral surveillance. Three-fourths of the SPHLs and one-third of the LPHLs provide on-site viral culture capacity.

PCR-based Molecular Assays

PCR-based molecular assays have afforded a dramatic improvement in diagnostic efficiency. Real-time PCR is widely accepted as a clinically useful test for diagnosing some infections: increased sensitivity and specificity, and speed of testing make this technology a preferred alternative to conventional PCR. However, for many pathogens, PCR is used in addition to, and not as a replacement for, culture. A majority (87%) of SPHLs and one-half (50%) the LPHLs provide PCR testing (on-site or sent out) for any of the bacterial pathogens. For any of the viral

agents, 78% of SPHLs and 43% of LPHLs report real-time PCR capacity (on-site or sent out).

DFA Testing

DFA, although presenting challenges to laboratories due to preparation of reagents, quality control, test performance and test interpretation subjectivity, has utility as a screening test for some pathogens. Of responding labs, 70% of the SPHLs and 36% of the LPHLs provide on-site or sent out DFA testing for any of the pathogens listed. Among antigen detection testing, on-site slide agglutination is performed by nearly 90% of SPHLs and 43% of LPHLs for any of the pathogens as listed in the following table.

Serologic Testing

Serologic testing, an adjunct to diagnosis by culture in many settings, can be especially useful when the suspected microbial agent either cannot be isolated in culture or can be isolated in culture only with great difficulty. An immunoassay is the most common serological testing procedure for demonstration of IgM and IgG antibodies. Few SPHLs (30%) or LPHLs (14%) provide on-site serologic testing for any of the bacterial organisms listed. Over three-fourths of SPHLs and one-half the LPHLs report providing this testing for any for the viral agents listed. One-half the SPHLs provide serologic testing for all the viral agents listed.

Conclusion

A timely public health response to outbreaks or unusual and rare cases of disease is dependent upon both state and local laboratory capabilities to isolate and identify bacterial and viral pathogens, which traditionally have been based upon the historical needs of the state. Changes in the epidemiology and control of vaccine-preventable and respiratory diseases require a detailed understanding of case characteristics and the pathogens that cause disease. For example, as the endemic transmission of certain VPDs is eliminated in the US, the risk for imported disease and outbreaks remains; public health laboratory case surveillance and detection becomes more critical for investigation into the sources of infection and epidemiologic chains of transmission. This survey provides an initial baseline assessment of the current capabilities for public health laboratory response to these challenges.

Background

Public health surveillance for respiratory and vaccine-preventable diseases requires close collaboration of clinicians, public health professionals and laboratorians. The nation's public health laboratories serve as part of the national surveillance infrastructure and have a rich tradition in applying cutting edge technology towards protecting the health of at risk populations. As part of this unique network, the Association of Public Health Laboratories (APHL) partners with laboratories to serve the public's health by promoting effective programs and public policy that support healthy communities, as well as by providing liaison between member laboratories and other key partners, including the Centers for Disease Control and Prevention (CDC). The capability of the public health laboratory system to provide a platform for diagnosis and surveillance is vital for the nation's response to emerging and existing infectious diseases.

Methods

To assess the volume of testing and current methodologies employed for a variety of vaccine-preventable and respiratory diseases and establish benchmarks for future comparison, APHL surveyed public health laboratories in August and September 2007. APHL member state and local public health laboratories were invited to participate in this survey, including those from the 50 states, the District of Columbia (DC) and four territories. A pre-tested, 41-question survey was self-administered using mrInterview, a Web-based data repository and survey tool. The invitational email described the survey and explained the study purpose. Survey non-responders received two reminder emails and one follow-up phone call from APHL. Laboratories were asked to respond to questions on current core laboratory testing capabilities and volumes (both on-site and sent out) for specified bacterial and viral pathogens or diseases. Survey data were exported into a SAS® (Statistical Analysis Software) file for subsequent analysis. Results are reported for the following testing methodologies and included the pathogens listed below. (See **Tables 1 and 2** for a test-specific pathogen list)

Overview of Testing Methodologies

- Culture
- Real-time PCR
- Conventional PCR
- DFA
- Serotyping or Serogroupoing
- Serologic

Bacteria

- *Bordetella* spp.
- *Chlamydia pneumoniae*
- *Chlamydia psittaci*
- *Clostridium tetani*
- *Corynebacterium diphtheriae*
- *Streptococcus pneumoniae*
- Group A *Streptococcus*
- *Haemophilus influenzae*
- *Legionella* spp.
- *Mycoplasma pneumoniae*
- *Neisseria meningitidis*

Viruses

- measles
- mumps
- rubella
- varicella

Results

Of 95 public health laboratories invited to participate, 68 (72%) responses were received. Of these, 46 state public health laboratory (SPHL) and 14 local public health laboratory (LPHL) surveys were complete and considered

valid for analysis; this report includes analysis (including missing or unknown data or responses) of these 60 completed surveys. **Tables 1 and 2** present summary data from the 60 surveys, while Appendices A-I provide pathogen-specific data.

Bacterial Testing

To assess capability for detecting and identifying specific bacterial pathogens, laboratories were asked to respond to a series of questions concerning diagnostic methodology provided "on-site" or "sent out" for "any" (one or more) or "all" (all listed for the testing platform). The diagnostic methodologies surveyed are listed above; Appendices A-E provide individual bacterial pathogen data. For summary bacterial data see **Table 1** on the next page.

Table 1. Public Health Laboratory Bacterial Testing Capacity

PROCEDURE	BACTERIA: *Bordetella* spp., *Clostridium tetani, Corynebacterium diphtheriae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia psittaci, Legionella* spp.	LABORATORY RESPONSE		
		ALL LABS N=60 YES %	SPHLS N=46 YES %	LPHLS N=14 YES %
Culture	Any† (on-site)	85	93	57
	All†† (on-site)	3	4	0
	Any (sent out)	62	65	50
	All (sent out)	10	4	29
PCR	**BACTERIA:** *Bordetella* spp., *Clostridium tetani, Corynebacterium diphtheriae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia psittaci, Legionella* spp., *Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae,* Group A *Streptococcus*			
	Any† (real-time, on-site or sent out)	78	87	50
	Any (conventional, on-site or sent out)	10	13	0
SEROLOGICAL TESTING	Any† (on-site)	27	30	14
	All†† (on-site)	3	4	0
DFA**	**BACTERIA:** *Bordetella pertussis, Bordetella parapertussis, Legionella spp.*			
	Any† (on-site or sent out)	62	70	36
	All†† (on-site or sent out)	12	15	0
SLIDE AGGLUTINATION	**BACTERIA:** *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*			
	Any† (on-site or sent out)	77	87	43

† = "ANY" indicates that this testing method is provided for at least one of the listed pathogens

†† = "ALL" indicates that this method is provided for all of the listed pathogens

NOTE: percentage calculations include missing/unknown data; results for individual pathogens are contained in Appendices A-E

Viral Testing

To assess capability for detecting and identifying specific viral pathogens, laboratories were asked a series of questions concerning diagnostic methodology provided "on-site" or "sent out" for "any" (greater than or equal to one) or "all" (all listed for the testing platform). **Table 2** below presents viral survey summary data; Appendices F-I present pathogen-specific data.

Discussion

The following section examines survey results for bacterial testing methodologies and pathogens as well as viral testing methodologies and pathogens.

Bacterial Testing Methodologies

Four general approaches are used for laboratory identification of bacteria: culture, PCR, serologic testing, DFA and slide agglutination.

Culture

Of the 60 laboratory responses, almost all (93%) of the SPHLs and over one-half (57%) of the LPHLs surveyed provide on-site culture for any of the bacterial pathogens listed (**Figure 1**). Many (65%) of the state public health and one-half (50%) of the local public health laboratories report sending out cultures for any of the bacterial pathogens listed, primarily for definitive identification or confirmation, sub-typing or additional diagnostic

Table 2. Public Health Laboratory Viral Testing Capacity

PROCEDURE	Viruses: measles, mumps, rubella, varicella-zoster	LABORATORY RESPONSE		
		All Labs N=60 Yes %	SPHLs N=46 Yes %	LPHLs N=14 Yes %
Culture	Any† (on-site)	67	76	36
	All†† (on-site)	18	20	14
	Any (sent out)	45	46	43
	All (sent out)	12	7	29
PCR	Any† (real-time, on-site or sent out)	70	78	43
	Any (conventional, on-site or sent out)	3	4	0
Serological Testing	Any† (on-site)	73	80	50
	All†† (on-site)	43	50	21
DFA**	Any† (on-site or sent out)	58	NA	NA

** VZ only

NA = not available

† = "ANY" indicates that this testing method is provided for at least one of the listed pathogens

†† = "ALL" indicates that this method is provided for all of the listed pathogens

NOTE: percentage calculations include missing/unknown data; results for individual pathogens are contained in Appendices F-I

Figure 1. Percent of Labs Providing Cultures On-Site and Sent Out

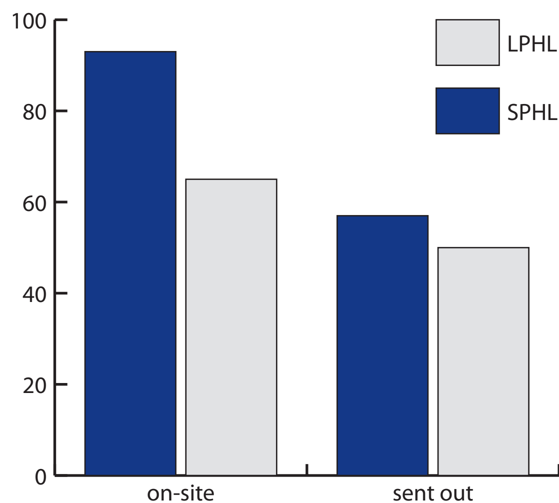
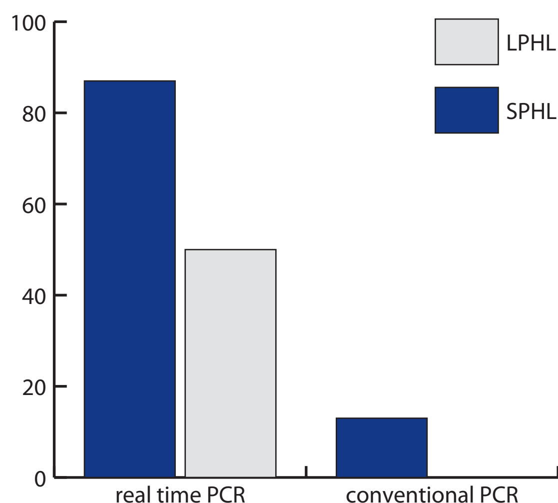


Figure 2. Percent of Labs Providing PCR (On-Site or Sent Out) by Methodology



procedures. **(Figure 1)**. Although the past three decades have witnessed growth of new technology in the diagnostic microbiology laboratory, culture is considered the gold standard and remains the most specific of the laboratory tests for many of the bacterial pathogens. Despite molecular methods providing increased sensitivity for identification of some organisms, culture retains epidemiological value in many settings. Decreased use of culture may be diminishing recognition of certain infections.

PCR

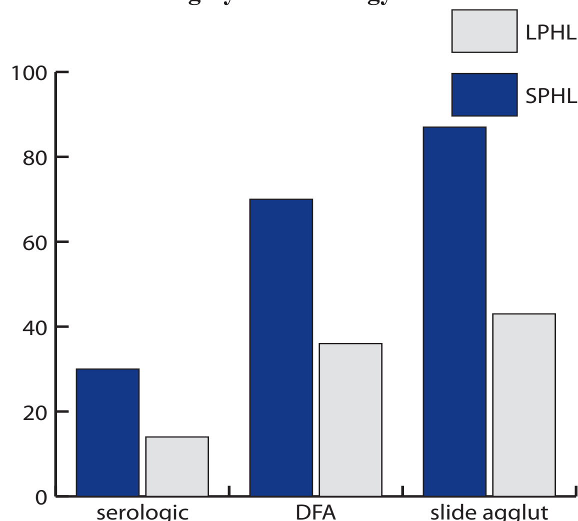
The majority (87%) of SPHLs and one-half (50%) the LPHLs **(Figure 2)** report the use of real-time PCR. Of the 60 laboratory responses, 13% of SPHLs and none of LPHLs report using conventional PCR for bacterial testing. Many laboratories now use polymerase chain reaction (PCR) molecular assays, which can provide faster reporting of results and increased sensitivity. PCR is widely accepted as a clinically useful test for diagnosing some infections (e.g., as pertussis). For the pathogens surveyed here, PCR is used in addition to, and not as a replacement for, culture.

Because of the combination of excellent sensitivity and specificity and speed, real-time PCR technology is often a preferred alternative to conventional PCR.

Serologic Testing

Few SPHLs (30%) or LPHLs (14%) report providing on-site serologic testing for any of the pathogens in this section (**Figure 3**). In many settings, serologic testing is considered an adjunct

Figure 3. Percent of Labs Providing On-Site or Sent Out Testing by Methodology



to diagnosis by culture; it is especially useful when the suspected microbial agent either cannot be isolated in culture by any known method or can be isolated in culture only with great difficulty.

DFA

DFA testing for any of the pathogens in this section **(see Table 1)** is provided by 70% of the SPHLs and 36% of the LPHLs **(Figure 3)**. Although presenting challenges to laboratories due to reagent preparation, quality control, test performance and test interpretation subjectivity, DFA has utility as a screening test in some settings.

Slide Agglutination

Of the 60 laboratory responses, nearly 90% of SPHLs report providing slide agglutination (serotyping or serogrouping) for *N. meningitidis, H. influenzae or Streptococcus pneumoniae,* either on-site or sent out. Fewer than one-half (43%) the LPHLs **(Figure 3)** report providing this test procedure for any of the pathogens in this section as listed in **Table 1**.

Bacterial Pathogens

This subsection considers survey results by bacterial pathogen.

Bordetella pertussis

Pertussis, a cough illness commonly known as whooping cough, is caused by the bacterium *Bordetella pertussis*. Three other *Bordetella* species cause disease in humans: *B. parapertussis*, *B. holmesii* and *B. bronchiseptica*. Routine childhood vaccination led to a reduction in disease incidence from an average of 150 reported cases per 100,000 persons between 1922 and 1940, to 0.5 per 100,000 in 1976. The incidence of reported pertussis began to increase in the 1980s; in 2005, the incidence of reported pertussis was 8.6 per 100,000 persons. The increasing circulation of *B. pertussis* among adolescents and adults, who are a major source of infection for infants, and the need for effective vaccine evaluation programs strengthen the need for reliable diagnostics.

The diagnosis of pertussis is complicated by the limitations of currently available diagnostic tests, with multiple factors affecting the sensitivity, specificity and interpretation of diagnostic tests. Since its inclusion of PCR in the case definition in 1997, the proportion of cases confirmed by this testing has increased, and PCR is now the most common method of case confirmation. Among responding labs, 83% of state labs and 54% of local labs report either performing onsite PCR for pertussis or sending samples out for this testing. However, culture capacity was reported for 93% of SPHLs and 62% of LPHLs. There are no standardized PCR assays for pertussis, with sensitivity and specificity varying greatly between laboratories. Consequently, CDC recommends that public health labs continue to culture pertussis cases and that suspected outbreaks are confirmed by culture.

Chlamydia pneumoniae

While the overall incidence of chlamydia pneumonia is unknown, *Chlamydia pneumoniae* is estimated to cause 2-5 million cases of pneumonia and 500,000 pneumonia-related hospitalizations each year in the United States. Chlamydia pneumonia is not currently a nationally notifiable condition.

Isolation of *C. pneumoniae* is difficult; therefore, most cases are identified by comparing acute and convalescent serum antibody titers. Efforts to standardize serologic testing for chlamydia pneumonia have been described elsewhere. Seven (13%) of the responding laboratories provide on-site serological testing for *C. pneumoniae*.

While some PCR assays are rapid, sensitive and specific for *C. pneumoniae*, these tests are not yet in widespread use. Real-time PCR is reported to be available (either on-site or referred out) in 9 (16%) of responding laboratories.

Chlamydia psittaci (Psittacosis)

Although outbreaks of psittacosis have been described frequently, since 1999, fewer than 25 cases have been reported to CDC each year. Diagnosis of psittacosis can be difficult, in part because a BSL-3 facility is necessary for culture. Six (11%) of 57 responding laboratories report providing on-site culturing, while 22 (39%) report being able to refer out for cultures.

Though antibiotic treatment may prevent an antibody response, antibody tests using paired acute- and convalescent-phase sera have been used for diagnosis. Seven (13%) of the responding laboratories indicate they provided on-site serological testing for *C. psittaci*.

While rapid and specific PCR assays are available for *C. psittaci*, the utility of this test for diagnosis in humans is unclear. Real-time PCR is reported to be available (either on-site or referred out) in1 (2%) of 55 responding laboratories.

Corynebacterium diphtheriae

Diphtheria is an uncommon disease in the US since the introduction and widespread use of vaccines. One case each was reported in 2002 and 2003. Isolation of *C. diphtheriae* on culture confirms infection, and most SPHLs provide culture on-site. A PCR test on clinical specimens to confirm infection with a toxigenic strain is available through the CDC. This test is useful if nonviable organisms are present in clinical specimens that are obtained after antibiotic therapy has been initiated. A case that is PCR positive without isolation of the organism, however, is not considered as laboratory confirmed.

Although measurement of a patient's serum antibodies to diphtheria toxin before administration of antitoxin may help in assessing the probability of the diagnosis of diphtheria, few laboratories have the capability to accurately test antibody levels. None of the survey respondents report this capacity.

Clostridium tetani

Documented tetanus incidence has declined in the US since the mid- to late 1940s, due primarily to the widespread use of vaccine, improved wound care management, use of tetanus prophylaxis in emergency rooms and decreased exposure to tetanus spores. The disease continues to occur almost exclusively among persons who are unvaccinated or inadequately vaccinated or whose vaccination histories are unknown or uncertain. In the period 2005-2006, 61 cases were reported in the US. The diagnosis of tetanus is entirely clinical as there are no characteristic laboratory findings. *C. tetani* is recovered from wounds in only about 30% of cases. Serological tests are rarely indicated, although results obtained before tetanus immune globulin (IG) is administered can support susceptibility under certain conditions.

Haemophilus influenzae

Invasive disease due to *H. influenzae* (Hi) is generally caused by one of six antigenically distinct capsular types (types a-f), but can also be caused by unencapsulated (nontypeable) strains. Prior to the introduction of conjugate vaccines for infants in 1990, Hi serotype b (Hib) was the cause of > 95% of invasive Hi diseases among children < 5 years of age with an incidence of nationally reported cases among children < 5 years of age of 41 cases per 100,000 in 1987. In the

first decade after the introduction of vaccine, incidence among children < 5 dropped to 1.6 cases per 100,000 in 2000.

All isolates from invasive cases in children < 5 need to be serotyped in order to continue monitoring vaccine effectiveness. All 40 (100%) of the responding SPHLs and 5 of the 13 (38%) LPHLs report the ability to perform agglutination testing. However, in 2007, serotype data was reported to CDC for < 70% of cases of nationally reported Hi cases among children < 5 years.

Legionella spp. (Legionellosis)

Between 8,000 and 18,000 hospitalized cases of Legionnaires' disease occur each year. The number of cases reported via national surveillance increased from 2,301 in 2005 to 2,834 in 2006. Efforts to improve surveillance for travel-associated Legionnaires' disease along with increased use of *Legionella* diagnostic methods may have contributed to this increase.

Identification of *Legionella* in respiratory secretions is challenging because of the difficulty in obtaining adequate respiratory specimens and because of the specialized medium and experience needed to isolate *Legionella.* Nevertheless, culture of respiratory specimens for *Legionella* is recommended in all suspected cases of Legionnaires' disease to guide antimicrobial therapy and allow matching of clinical cases to environmental sources. Routine on-site clinical culture was reported to be available at 23 (40%) of 58 responding laboratories; an additional 14 (24%) by special request.

Despite the value of culture of respiratory specimens, the most commonly used diagnostic test for Legionnaires' disease is the *Legionella* urinary antigen test. Available commercially, it is a rapid and convenient diagnostic tool; however, it only detects *Legionella pneumophila* serogroup 1, the most common pathogenic species and serogroup of *Legionella*. In situations where Legionnaires' disease is suspected, both culture and urinary antigen should be considered.

Because the reservoirs for *Legionella* are aquatic environments, most legionellosis outbreak investigations require a substantial environmental component. Environmental water and biofilm samples may be collected to detect the presence of *Legionella* in the suspected water source. Twenty-six (45%) laboratories reported having the capability to isolate *Legionella* from environmental water samples.

Mycoplasma pneumoniae

Mycoplasma pneumoniae is estimated to cause between 2 million cases of pneumonia and 100,000 pneumonia-related hospitalizations each year in the United States. Mycoplasma pneumonia is not currently a nationally notifiable condition.

In clinical practice, most cases are diagnosed by serology. Serologic testing for presence of IgM antibody or paired serology for combined IgM/IgG is available. On-site serologic testing is reported to be available at 7 (13%) of the reporting laboratories. PCR might be superior to serology for diagnosis of acute illness; however, it is not widely utilized clinically. Real-time PCR testing (either on-site or referred out) is available at 10 (18%) laboratories.

Neisseria meningitidis

Meningococcal disease is a serious and potentially life-threatening infection caused by the bacterium *Neisseria meningitidis*. Approximately 1,400 – 2,800 cases of meningococcal disease occur annually in the United States, a rate of 0.5-1.1/100,000 population. *N. meningitidis* became one of the leading causes of bacterial meningitis in the United States following the reduction in incidence of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b due to the introduction of conjugate vaccines for these pathogens.

N. meningitidis can be classified into 13 serogroups based on the immunologic reactivity of their capsular polysaccharides. Serogroups B, C and Y each cause approximately one-third of meningococcal disease cases in the United States. The proportion of cases caused by each serogroup varies by age; serogroup B causes over 50% of cases in children < 1 year while serogroups C, Y and W135 cause 75% of meningococcal disease in those ≥ 11 years. All 40 (100%) of the responding state public health labs and 5 (38%) of the 13 local public health labs reported ability to distinguish serogroups. However, in 2007, serogroup data was reported to CDC for less than half of cases reported through national surveillance.

Streptococcus pneumoniae (pneumococcus)

Streptococcus pneumoniae is a common cause of otitis, pneumonia, bacteremia and meningitis worldwide. Of the 91 pneumococcal serotypes, seven are included in pneumococcal conjugate vaccine (PCV7, Prevnar®) and 23 are included in the pneumococcal polysaccharide vaccine (PPV23, Pneumovax®). With the introduction of PCV7 for use in children less than five years of age in 2000, rates of invasive pneumococcal disease in that group declined by 77 percent. Rates of disease have also declined among older age groups as a result of decreased colonization in and transmission from children. A new conjugate vaccine formulation that targets 13 serotypes is expected to replace PCV7 in 2009.

S. pneumoniae PCR tests are available for upper respiratory specimens; however, as with all diagnostic tests for pneumococcus on upper respiratory specimens, they are unable to distinguish colonization from infection. Three (5%) laboratories report having the ability to perform real-time PCR for *S. pneumoniae* (either on-site or referral).

Because PCV7 includes only seven pneumococcal serotypes, serotyping of *S. pneumoniae* can be used to track the impact of PCV7 programs. Classical serotyping is expensive and labor-intensive and, therefore, not widely available. Conventional PCR-based serotyping assays are currently available (http://www.cdc.gov/ncidod/biotech/strep/pcr.htm) and a multiplex real-time PCR assay is under development. Among responding laboratories, 6 (12%) indicated on-site availability of serotyping capacity.

Viral Testing Methodologies

Three general approaches are used for laboratory diagnosis of viral infections: 1) isolation of the virus (culture); 2) direct detection of viral components, either in cells derived from infected patient specimens or by amplification of viral genes by PCR from nucleic acid extracted from patient samples; and 3) demonstration of a significant increase in IgG antibody levels or the presence of IgM antibody (serology).

Culture

As shown in **Figure 4**, 76% of the SPHLs and 36% of the LPHLs report providing on-site viral isolation. Viral isolation, although not the test of choice for rapid clinical diagnosis for the pathogens included in this survey, is an important and essential tool for epidemiologic viral surveillance. Of the 60 laboratory responses, both SPHLs (46%) and LPHLs (43%) report sending out viral cultures, most often to the CDC for viral isolation, confirmation, or for genotyping. With today's emphasis on molecular techniques, viral culture is becoming less common, although culture remains the gold standard for isolation of important pathogens.

Figure 4. Percent of Labs Providing On-Site or Sent-Out Viral Cultures

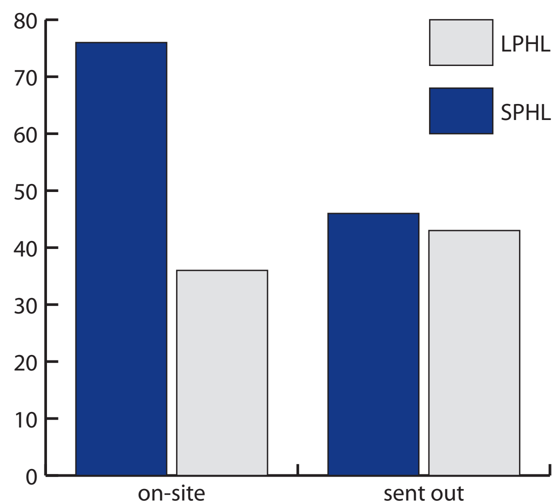
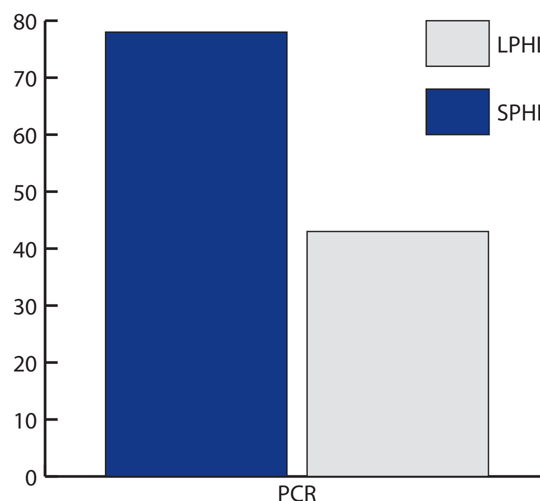


Figure 5. Percent of Labs Providing On-Site or Sent Out Viral PCR Testing



PCR

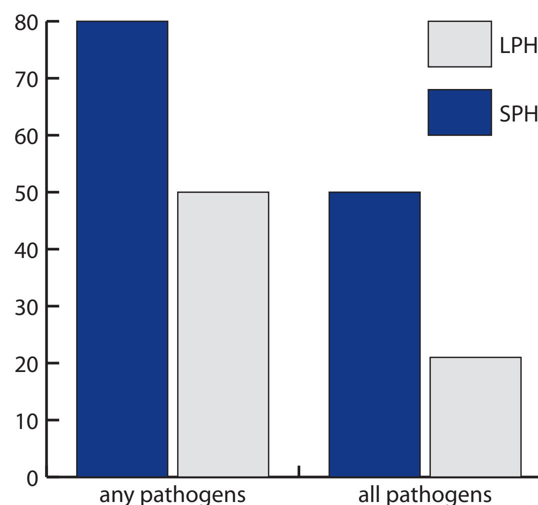
Of the SPHLs, 78% provide either on-site or sent out PCR testing; of the LPHLs, 43% indicate providing this testing either on-site or sent out (**Figure 5**). Real-time PCR from clinical specimens has improved the ability to provide viral detection. Due to the increased sensitivity of real-time PCR, it is likely that some samples that are real-time positive cannot be amplified using conventional PCR. Therefore, viral culture may still be necessary in order to provide sufficient viral RNA necessary for genotyping.

Conventional PCR and sequence analysis of viruses play an important role in surveillance of viral agents for diseases that occur infrequently or are no longer endemic in the US to determine virus origin and circulating viral strains. Genotyping can be used to distinguish between wild-type and vaccine-type virus.

Serologic Testing

As shown in **Figure 6**, over three-fourths of SPHLs and one-half the LPHLs provide on-site serologic testing for any of the viral pathogens in this section **(see Table 2)**. One-half of the SPHLs (data not shown) provide serologic testing for all the viruses listed.

Figure 6. Percent of Labs Providing On-Site Serologic Testing



Serologic testing for the demonstration of IgM and IgG antibodies is widely used, and the enzyme immunoassay (EIA) is the most common format (data not shown) among the survey participants. Because viral isolation and genetic characterization can take several weeks to complete, laboratory diagnosis of viral diseases is often based on detection of virus-specific IgM in a single serum sample.

Viral Pathogens

This subsection addresses survey results by viral pathogen.

Measles Virus

Due to high vaccination coverage, measles has not been endemic in the US since 2000, although outbreaks associated with importations continue to occur. During the ten years (January 1, 1998 through December 31, 2007), 703 total cases were reported nationally, ranging from 100 in 1998 to 37 in 2004. Measles elimination and control programs depend on effective viral surveillance, which when used with epidemiologic and molecular data, can assist in documenting the elimination of endemic transmission. Virus isolation, therefore, is an extremely important microbiologic tool for investigating cases.

Primary monkey kidney (PMK) cells are often used for measles virus (MV) isolation from patients, but the success rate is variable and viral culture can take several days to weeks to complete. Consequently, culture is not used as the primary means for measles diagnosis. Of the survey participants responding to this question, 40% indicate they perform on-site viral culture for MV routinely, while another 7% do culture selectively; PMK cells were used by a majority of these labs. Specimens are sent out for MV testing by 15% of the labs. IFA is the most commonly reported methodology for antigen detection, followed by DFA. No labs reported using PCR to confirm virus isolation. Identification of the viral genotype by RT-PCR and sequence analysis can take weeks to complete but the information is an important tool for molecular epidemiologic surveillance.

Serologic testing (IgM/IgG antibody) for MV is widely used: 64% of responding laboratories report performing this testing on-site using a commercial assay. An enzyme-linked immuno-assay (EIA) test for IgM antibody, requiring only a single serum specimen, is diagnostic if positive; 85% of responding laboratories report using either a capture (the preferred reference test) or indirect EIA for IgM. Testing for IgG is often provided along with IgM testing; 48% of responding laboratories report providing IgG testing. Testing sent out, mainly for IgM, is reported by 37% of the labs. Qualitative (81%) IgG testing is performed more often than quantitative (19%) testing.

Mumps Virus

The reported incidence of mumps in the US declined following the introduction of mumps vaccine in 1967 and the recommendation for its routine use in 1977. Expanded recommendations for a 2-dose MMR vaccine schedule for measles control in 1989 precipitated a further decline in mumps cases. Approximately 10,000 cases, ranging from 231 in 2003 to 800 in 2007, were reported in the US from January 1, 1998, to December 31, 2007. A large multi-state outbreak in 2006 resulted in 6,582 reported cases, the largest number of mumps cases for a single year since 1987, when 12,848 cases were reported; fewer than 300 cases had been reported each year from 2001 through 2004. Epidemiologic and molecular data are critical for tracking transmission pathways of mumps strains circulating in the United States and to distinguish wild-type mumps virus from vaccine strain virus.

Mumps virus is cultured in a variety of primary and continuous cell lines; isolation of the virus from a patient establishes a diagnosis of mumps. As demonstrated in recent outbreaks, there is a lack of a reliable IgM response among vaccinated persons who become infected. As a result, it has become more important to test samples by culture and/or PCR as a means of

diagnosis. Routine on-site culture for mumps virus is reported by 51% of respondents; PMK is the cell line of choice for the majority of survey respondents. Of the responding labs, 30% report sending out viral specimens for mumps culture. IFA, relatively simple and inexpensive, is the most commonly reported methodology for antigen detection to confirm the presence of virus in culture, followed by PCR. RT-PCR can be used to detect mumps RNA directly from clinical samples as well as to confirm the presence of virus in cell culture inoculated with the clinical sample.

Few laboratories (25%) report performing on-site serological testing for mumps IgM antibody, the majority using either IFA or EIA indirect assays, while 45% report performing IgG antibody testing (the majority using an indirect EIA assay). Testing sent out, mainly for IgM, is reported by 35% of the labs. Qualitative (71%) IgG testing is performed more often than quantitative (29%) testing.

Rubella Virus

Since 2004, rubella has been considered no longer endemic in the US, although cases due to importation are reported each year. During the ten years (January 1, 1998 through December 31, 2007), 899 total cases were reported nationally, ranging from 364 in 1998 to 7 in 2003. Virologic and epidemiologic surveillance provide evidence of the interruption of endemic transmission of rubella virus in the US, but must be joined with active vaccination programs as long as the threat of disease importation exists. Rubella culture, which provides isolates for molecular typing, is very important for epidemiologic surveillance.

Although virus culture is rarely used as laboratory confirmation of rubella cases, virus isolates are very important for surveillance. Primary African Green Monkey Kidney (AGMK) cells are considered the standard for isolation of rubella virus from clinical specimens, although a wide variety of cell types are susceptible to infection by rubella virus. Routine on-site rubella virus culture is reported by only 11% of respondents; PMK is the cell line of choice for the majority of survey respondents. Of labs responding, 39% report sending viral specimens out for testing, most often to the CDC. The majority of respondents report using IFA or DFA for antigen detection (culture confirmation); no labs report using PCR.

Serologic techniques for the detection of antibodies to rubella virus provide laboratory diagnosis of acute infections and the ability to determine rubella immune status. Methods include hemagglutination inhibition (HI), enzyme immunoassay (EIA), IFA, CF and a variety of rubella-specific IgM antibody assays. Of responding labs, 48% report on-site testing for rubella IgM antibody, with either the capture (37%) or indirect (48%) EIA assay used most often. IgG antibody testing is reported by 57% of responding labs with an EIA indirect assay used more frequently. Of labs responding to testing sent out, 33% report sending out specimens, mainly for IgM testing. Qualitative (84%) IgG testing is performed more often than quantitative (26%).

Varicella-Zoster (VZ) Virus

A varicella vaccine was licensed in 1995 with recommended use in children – infants 12-18 months of age, as well as susceptible older children, adolescents and adults, since varicella affected mainly children with 90% of cases occurring before the age of 15 years. Although increased vaccination of children has lowered the overall burden of disease, a higher proportion of cases occur among vaccinated persons (i.e., vaccine failure), in which the illness is typically mild and presents with a modified rash that can

be hard to recognize and diagnose. In addition, a higher proportion of cases is expected to occur in the older children, adolescents and young adults who may have escaped the disease or vaccination. Although national disease surveillance is limited due to only a few states now reporting, surveillance data document a decline in disease incidence in certain age groups.

Viral culture is very specific but it is insensitive and labor intensive. It can, however, be useful when other diagnostic testing platforms are not available. Of responding labs, 60% report routine on-site viral culture for varicella; 9% report performing culture based on specific criteria.

Detection of VZ viral DNA in specimens from skin lesions using PCR is a sensitive, rapid and versatile tool for diagnosing VZ infection. Of responding labs, 68% report using real-time PCR testing; no labs report using conventional PCR.

DFA testing is widely available and very specific, though its sensitivity is highly dependent on proper sample collection technique. On-site DFA testing for varicella is reported by 58% of responding laboratories.

Serologic procedures for IgM and IgG can be used for laboratory diagnosis of varicella, however IgM testing is not very sensitive, and IgG testing requires acute and convalescent serum specimens making it impractical in many settings. While IgG testing can be used to determine whether or not there had been a history of varicella disease, routine tests are not sufficiently sensitive to detect varicella-vaccine mediated IgG. Of labs responding to providing on-site serology, either IgM or IgG or both, 57% report this testing; 26% report sending specimens out for this testing.

Summary

A state's response to outbreaks or unusual and rare cases of disease is dependent upon both state and local laboratory capabilities to isolate and identify bacterial and viral pathogens, which have traditionally been based upon the historical needs of the state. Changes in the epidemiology and control of vaccine-preventable and respiratory diseases require a detailed understanding of case characteristics and the pathogens that cause disease. For example, as the endemic transmission of certain VPDs is eliminated in the US, the risk for imported disease and subsequent outbreaks remains; public health laboratory case surveillance and detection becomes more critical for investigation into the sources of infection and epidemiologic chains of transmission. This survey provides an initial baseline assessment of current capabilities for public health laboratory response to these challenges.

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Appendix A

Bacterial Cultures Provided (On-site or Sent Out)

<table>
<tr><th rowspan="3">Organism</th><th rowspan="3">Current Concepts on Culture</th><th colspan="2">Laboratory Response*</th></tr>
<tr><th>SPHLs</th><th>LPHLs</th></tr>
<tr><th>Yes # (%)</th><th>Yes # (%)</th></tr>
<tr><td rowspan="4">Bordetella spp.</td><td rowspan="4">Isolation of B. pertussis or parapertussis by bacterial culture is the standard and preferred diagnostic laboratory test: a positive culture confirms the diagnosis. However, culture is relatively insensitive and the organism is difficult to isolate.</td><td colspan="2">Cultures; N=56 on-site</td></tr>
<tr><td>40 (71)</td><td>8 (14)</td></tr>
<tr><td colspan="2">Cultures; N-55 sent out</td></tr>
<tr><td>2 (4)</td><td>3 (3)</td></tr>
<tr><td rowspan="4">Clostridium tetani</td><td rowspan="4">There are no laboratory findings characteristics of tetanus; diagnosis is entirely clinical and does not depend upon bacteriologic confirmation. C. tetani is recovered from wounds in only 30% of cases.</td><td colspan="2">Cultures; N=55 on-site</td></tr>
<tr><td>21 (38)</td><td>4 (7)</td></tr>
<tr><td colspan="2">Cultures; N-56 sent out</td></tr>
<tr><td>16 (29)</td><td>4 (7)</td></tr>
<tr><td rowspan="4">Corynebacterium diphtheriae

(N=56)</td><td rowspan="4">A bacteriological culture is essential for confirming diphtheria. However, requests are rare and isolation requires special selective short shelf life media. There are no other commercially available tests for diagnosing diphtheria.</td><td colspan="2">Cultures on-site</td></tr>
<tr><td>37 (66)</td><td>6 (11)</td></tr>
<tr><td colspan="2">Cultures sent out</td></tr>
<tr><td>10 (18)</td><td>3 (5)</td></tr>
<tr><td rowspan="4">Chlamydia pneumoniae

(N=57)</td><td rowspan="4">The recommended procedure for primary isolation of Chlamydiae is culture, which is important for confirming diagnosis in selected cases but may not be necessary for treatment or public health investigations.</td><td colspan="2">Cultures on-site</td></tr>
<tr><td>6 (11)</td><td>0 (0)</td></tr>
<tr><td colspan="2">Cultures sent out</td></tr>
<tr><td>15 (26)</td><td>6 (11)</td></tr>
<tr><td rowspan="4">Chlamydia psittaci

(N=57)</td><td rowspan="4">This is a BSL-3 agent that requires an appropriate handling, processing and testing facility. All chlamydiae (with some variability) will grow in cell culture, which is the primary procedure for isolation.</td><td colspan="2">Cultures on-site</td></tr>
<tr><td>6 (11)</td><td>0 (0)</td></tr>
<tr><td colspan="2">Cultures sent out</td></tr>
<tr><td>16 (28)</td><td>6 (11)</td></tr>
<tr><td rowspan="4">Legionella spp.

(N=58)</td><td rowspan="4">Isolation of Legionella from culture is highly desirable for public health investigations as it allows matching of clinical cases to environmental sources.</td><td colspan="2">Cultures on-site</td></tr>
<tr><td>32 (55)</td><td>5 (9)</td></tr>
<tr><td colspan="2">Cultures sent out</td></tr>
<tr><td>16 (28)</td><td>6 (10)</td></tr>
<tr><td rowspan="4">Mycoplasma pneumoniae

(N=56)</td><td rowspan="4">Detection by culture requires several days for growth and is not always successful, but isolation confirms infection.</td><td colspan="2">Cultures on-site</td></tr>
<tr><td>3 (5)</td><td>0 (0)</td></tr>
<tr><td colspan="2">Cultures sent out</td></tr>
<tr><td>15 (27)</td><td>6 (11)</td></tr>
</table>

*Missing data are excluded in the percent calculations—see total number (N=) with available data

Appendix B

Real-Time PCR Provided (On-site or Sent Out)

Organism	Current Concepts on PCR	Laboratory Response*	
		SPHLs	LPHLs
		Yes # (%)	Yes # (%)
Bordetella pertussis (N=55)	PCR is rapid, sensitive and specific; it is often used to confirm culture. However, this method should be used alongside culture.	35 (64)	7 (13)
Bordetella parapertussis (N=54)	PCR is rapid, sensitive and specific; however, this method should be used in addition to culture.	5 (9)	11(20)
Corynebacterium diphtheriae (N=54)	PCR testing, which is provided by reference laboratories such as the CDC, is useful if nonviable organisms are present in clinical specimens.	4 (7)	0 (0)
Chlamydia pneumoniae (N=55)	Some PCR assays are rapid and specific. A test is available but not widely used.	9 (16)	0 (0)
Chlamydia psittaci (N=55)	Some PCR assays are rapid, sensitive and specific. A test is available but not widely used.	1 (2)	0 (0)
Legionella spp. (N=56)	A PCR assay is available but clinical and public health utility has not been studied.	10 (18)	0 (0)
Mycoplasma pneumoniae (N=56)	A rapid, sensitive and specific PCR test is available.	10 (18)	0 (0)
Neisseria meningitidis (N=57)	Real time PCR detects DNA of meningococci in blood, CSF, or other clinical specimens, which is especially important for cases in which the organism could not be detected by culture methods.	5 (9)	3 (5)
Haemophilus influenzae (N=54)	PCR assays for Hib are available for research purposes only.	3 (6)	0 (0)
Streptococcus pneumoniae (N=55)	PCR tests are available for upper respiratory specimens but are unable to distinguish colonization from infection.	3 (5)	0 (0)
Group A *Streptococcus* (N=55)	GAS PCR is not performed routinely and is typically available only in reference and research labs.	4 (8)	0 (0)

*Missing data excluded in percentage calculations—see total number (N=) with available data

Appendix C

Serologic Testing Provided On-site

Organism	Current Concepts on Serologic Testing	Laboratory Response*	
		SPHLs	LPHLs
		Yes # (%)	Yes # (%)
Bordetella spp.	Standardized serologic tests to diagnose pertussis are not available.		
Clostridium tetani	Serological results obtained before tetanus IG is administered can be used to determine patient susceptibility.		
Corynebacterium diphtheriae	Measurement of patient serum antibodies before administration of antitoxin may help in the diagnosis of diphtheria, although few laboratories have the capability to accurately test antibody levels.		
Chlamydia pneumoniae (N=54)	Antibiotic treatment may prevent antibody response, thus limiting diagnosis by serologic methods. Fourfold or greater increase in antibody against *C. pneumoniae* by complement fixation (CF) or microimmuno-fluorescence (MIF) to a reciprocal titer of greater than or equal to 32 between paired acute- and convalescent-phase serum specimens is diagnostic.	7 (13)	0 (0)
Chlamydia psittaci (N=55)	Antibiotic treatment may prevent antibody response, thus limiting diagnosis by serologic methods. Fourfold or greater increase in antibody against *C. psittaci* by CF or MIF to a reciprocal titer of greater than or equal to 32 between paired acute- and convalescent-phase serum specimens is diagnostic.	6 (11)	1 (2)
Legionella spp. (N-55)	A four-fold increase between acute and convalescent sera is required to confirm diagnosis. A single high serum titer is not diagnostic.	12 (22)	0 (0)
Mycoplasma pneumoniae (N=56)	Serological testing for presence of IgM antibody or paired serology for combined IgM/IgG is available.	7 (13)	0 (0)
Neisseria meningitidis	Antibody testing is not available.		
Haemophilus influenzae	Antibody testing is not available.		
Streptococcus pneumoniae	Serological testing is not available for *Streptococcus pneumoniae.*		
Group A *Streptococcus* (N=55)	M protein type-specific antibody testing, which is occasionally performed in research labs, is not done routinely and is labor-intensive.	0 (0)	0 (0)

*Missing data excluded in percentage calculations—see total number (N=) with available data

Appendix D

DFA Provided (On-site or Sent Out)

Organism	Current Concepts on DFA	Laboratory Response*	
		SPHLs	LPHLs
		Yes # (%)	Yes # (%)
Bordetella pertussis (N=54)	DFA has low sensitivity and variable specificity when testing nasopharyngeal specimens; it may be useful, however, as a screening test and should be used alongside culture or PCR.	19 (35)	1 (2)
Bordetella parapertussis (N=54)	DFA has low sensitivity and variable specificity when testing nasopharyngeal specimens; it may be useful for screening and should be used alongside culture or PCR.	12 (22)	0 (0)
Legionella spp. (N=54)	Due to low specificity, other methods are preferred.	20 (36)	1 (2)

*Missing data excluded in percentage calculations—see total number (N=) with available data

Appendix E

Serotyping or Serogrouping Provided (On-site or Sent Out)

Organism	Current Concepts on Serotyping or Serogrouping	Laboratory Response*	
		SPHLs	LPHLs
		Yes # (%)	Yes # (%)
Streptococcus pneumoniae (N=52)	Classical serotyping, which is performed in a limited number of state PH labs, is labor-intensive and costly; a multiplex real time PCR assay is under development.	14 (27)	2 (4)
Neisseria meningitidis (N=52)	Slide agglutination serogrouping is widely available. Latex agglutination is used for rapid detection of meningococcal capsular polysaccharides in CSF.	40 (75)	5 (9)
Haemophilus influenzae (N=53)	Serotyping distinguishes encapsulated strains, including Hib, from unencapsulated strains; testing kits are widely available.	40 (75)	2 (4)

*Missing data excluded in percentage calculations—see total number (N=) with available data

Appendix F

Viral Cultures Provided (On-site or Sent Out)

Disease	Current Concepts on Culture	Laboratory Response*	
		SPHLs Yes # (%) N = 57	LPHLs Yes # (%)
Measles	Due to poor sensitivity, isolation of the virus is not recommended as a method for diagnosis. However, if positive, these tests can be useful adjuncts to diagnosing acute measles when serology is inconclusive. Isolates are important for molecular epidemiologic reasons.	**Cultures on-site**	
		24 (42)	3 (5)
		Cultures sent out	
		12 (21)	4 (7)
Mumps	Virus culture is rarely performed for diagnosis of uncomplicated cases. Successful isolation is usually confirmed by IF or by molecular techniques. Isolates are important for surveillance: molecular typing of isolates provides epidemiologically important information.	**Cultures on-site**	
		24 (42)	3 (5)
		Cultures sent out	
		10 (18)	6 (11)
Rubella	Virus culture is recommended, although it is rarely used as lab confirmation of cases because it is slow and expensive. Isolates are important for surveillance: molecular typing of isolates provides epidemiologically important information.	**Cultures on-site**	
		10 (18)	2 (4)
		Cultures sent out	
		18 (32)	4 (7)
Varicella-Zoster	The diagnosis of VZV infection may be confirmed by culture. The virus is difficult to isolate, but culture can be useful when other diagnostic testing platforms are not available.	**Cultures on-site** ALL LABS N=57	
		34 (60)	
		Cultures sent out ALL LABS N=55	
		7 (13)	

* Missing data excluded in the percent calculations – see total number (N=) with available data

Appendix G

Real Time PCR Provided (On-site or Sent Out)

Disease	Current Concepts on PCR	Laboratory Response*	
		SPHLs	LPHLs
		Yes # (%)	Yes # (%)
Measles (N=55)	RT-PCR is important for molecular epidemiologic surveillance to determine virus origin, circulating viral strains and whether the strain has become endemic in the US. The viral genotype can be used to distinguish between wild-type and vaccine virus.	9 (16)	1 (2)
Mumps (N=54)	Molecular epidemiologic surveillance makes it possible to track transmission pathways of mumps strains circulating in the US, as well as to distinguish wild-type from vaccine virus. Detection of virus by RT-PCR confirms a diagnosis of mumps.	20 (37)	1 (2)
Rubella (N=54)	Detection of rubella virus is often necessary to confirm rubella cases and characterize wild-type rubella viruses. RT-PCR is used to confirm sporadic cases and outbreaks of rubella. Clinical specimens for virus isolation and sent to CDC are screened by RT-PCR.	2 (4)	0 (0)
Varicella-Zoster (N=56)	PCR testing for varicella zoster virus DNA in specimens collected from skin lesions is a sensitive, rapid and versatile tool for diagnosing varicella.	**ALL LABS**	
		38 (68)	

*Missing data excluded in percentage calculations—see total number (N=) with available data

Appendix H

Serologic Testing Provided On-site

Disease	Current Concepts on Serologic Testing	Laboratory Response*	
		SPHLs	LPHLs
		N = 56	
Measles	An EIA, the most consistently accurate test for IgM antibody, is the recommended method for diagnosis of acute disease. A single-specimen test for IgG antibody is most commonly used to determine immunity. Paired sera to detect a four-fold rise in IgG antibody is used to confirm a diagnosis.	Yes # (%)	Yes # (%)
		32 (57)	4 (7)
Mumps	Available serologic tests include an EIA, a highly specific test for diagnosing acute infection, which is confirmed by the presence of mumps IgM or a significant rise in IgG antibody titer. IFA assays, relatively inexpensive and simple, are available commercially but are not FDA approved. Serologic testing has been found to be unreliable in diagnosing infection in previously vaccinated individuals.	Yes # (%)	Yes # (%)
		30 (54)	3 (5)
Rubella	Serologic tests available for screening for immunity and confirmation of disease include an EIA, the preferred method for IgM antibody. Once the gold standard, Hemagglutination Inhibition (HI) allows for screening or confirmation of infection. Latex Agglutination (LA) is a very rapid, sensitive and specific test for screening. A rapid and sensitive assay, Immunofluorescent Antibody (IFA) is available commercially for both IgM and IgG.	Yes # (%)	Yes # (%)
		33 (59)	7 (12)
Varicella-Zoster	Serologic testing can be used to detect IgG directed against varicella zoster virus and determine whether or not there had been a history of varicella disease. IgG testing is, however, not sufficiently sensitive to detect vaccine-mediated IgG. Although acute varicella can be confirmed serologically, IgM testing is insensitive, and a rise in IgG titers is often impractical since it requires collection of acute and convalescent serum specimens.	Serology On-Site # (%) ALL LABS N=56	
		32 (57)	

*Missing data excluded in percentage calculations—see total number (N=) with available data

Appendix I

DFA Provided On-Site

Organism	Current Concepts on DFA	Laboratory Response*	
		SPHLs	LPHLs
		Yes # (%)	Yes # (%)
Varicella-Zoster	DFA testing is widely available and specific, though its sensitivity is highly dependent on proper sample collection technique.	**DFA on-site** **# (%)** ALL LABS N=55	
		32 (58)	

*Missing data excluded in percentage calculations—see total number (N=) with available data

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