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We thank the CDC/NCIRD subject-matter experts and the state public health laboratory representatives who helped to generate questions for this survey and to those who participated in developing the training activities based on the interpretation of the collected survey responses.

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APHL MISSION
To promote the role of public health laboratories in shaping national and global health objectives, and to promote policies, programs, and technologies that assure continuous improvement in the quality of laboratory practice and health outcomes.
Executive Summary

Background
This survey was done to provide information on public health laboratory (PHL) testing capabilities, training needs, proficiency testing gaps, and on the value of PHL Centers of Excellence (COEs). The responses from this survey are intended to guide the development of further training and quality improvement activities as stated in the VPD project scope of work. This work was supported by a grant received by the Association of Public Health Laboratories (APHL) in September 2009 under the American Recovery and Reinvestment Act of 2009 (ARRA). The information from the survey, which was done in collaboration with the U.S. Centers for Disease Control and Prevention (CDC), will be used to improve the diagnostic testing capabilities of PHLs for Vaccine-Preventable Diseases (VPD).

Following the delivery of an explanatory email, a survey was distributed electronically to the ninety-four current APHL member state and local public health laboratories. The survey questions were entered into the online MRInterview survey tool. The questions addressed laboratory testing for nine bacterial and viral pathogens that are causative agents for Vaccine-Preventable Diseases: Bordetella pertussis; Streptococcus pneumoniae; Neisseria meningitidis; Haemophilus influenzae; rotavirus; and measles, mumps, rubella, and varicella-zoster viruses. There were also general questions for Laboratory Directors and upper management personnel on proficiency testing, training and the potential implementation of regional “Centers of Excellence” laboratories for VPD reference testing.

Response
Of the ninety-four APHL member public health laboratories contacted, sixty-eight responded, for an overall response rate of 72%. Nine of these respondents indicated they do not test for any of the listed pathogens and were thereby not required to respond to the survey questions nor included in any of the assessed data. The subsequent survey analysis is based on the completed surveys submitted by the 59 laboratories where testing for some or all of the listed pathogens is performed, providing an applicable response rate of 63%. This response group included 15 (out of 40) Local Public Health Laboratories (LPHL) and 44 (out of 54) State Public Health Laboratories (SPHL) which were APHL member labs at that time.

RESULTS SUMMARY

Bacterial Testing
- Culture and biochemical testing are used by all laboratories for at least one bacterial VPD.
- PCR is often used for B. pertussis identification (75%), more often than PCR is used to identify the other three bacterial agents addressed in the survey.
- Twelve different gene targets are used in various combinations by laboratories for their Pertussis PCR assays.
- Slide agglutination is often used for typing of H. influenzae (68%) and N. meningitidis (85%); but not for S. pneumoniae (2%).
- 83% of laboratories do not perform PCR-based serogrouping/serotyping for these three bacterial agents.

Viral Testing
- 68% of laboratories do not perform rotavirus testing by any of the methods included in the survey: culture, EIA serology testing, and PCR.
- Most laboratories perform testing to detect at least one of the MMRV viruses (measles, mumps, rubella, and varicella-zoster) by serology (83%) and/or culture (71%).
- 42% of laboratories perform PCR testing for at least one of the MMRV viruses by a non-LRN (Laboratory Response Network) PCR method. An additional 36% of laboratories perform only the LRN PCR assay for varicella-zoster.
- 44% of laboratories indicate that they do not have sufficient information on assay performance to properly evaluate the commercially available ELISA and PCR kits for the viral agents.
Testing Capability
- Responding laboratories listed the greatest deterrent to implementing new testing methods for VPDs as low testing volume.
- Funding and staffing shortages are also significant factors for lack of testing capability.

Proficiency Testing (PT):
- PT panels for VPDs are not perceived to be sufficient by many of the laboratories for their proficiency testing needs, and they indicated that they have a lack of information on PT panel options for VPDs.
- For each of the pathogens in the survey, a majority (81-100%) of responding laboratories do not currently participate in sample sharing programs with other laboratories for QA or proficiency testing purposes. Some laboratories requested assistance with specimen exchange programs.

Training Needs
- Up to one-third to one-half of the responding laboratories indicated an interest in teleconferences and self-study web-based trainings for all testing methods included in the survey.
- Topics of interest for both web-based training and teleconference trainings were the same and included: QC/troubleshooting/difficult case studies for meningococcus and H. influenzae slide agglutination, Multiplex PCR for pertussis and PCR for measles, mumps, rubella, and varicella-zoster viruses.
- Topics of interest for hands-on laboratory workshops were divided into two groups, bacterial VPDs and viral VPDs and can be seen in List 5.

Centers of Excellence
- 95% of laboratories indicated that they would be interested in utilizing regional “Centers for Excellence” laboratories for at least some reference testing that they do not perform themselves.
- The major barriers to utilizing regional testing centers were shortages in staffing (83%) and funding for both specimen shipments (73%) and reagents/controls (63%).
- A majority (54%) of the respondents indicated they could serve as a regional COE for VPD reference testing for at least one of the pathogens in the survey if sufficient funding and support were provided.

CONCLUSION
The responses of the 59 public health laboratories surveyed in this assessment reveal the need for a number of training and quality improvement initiatives for VPD testing in PHLs. These initiatives include teleconferences and hands-on trainings, proficiency testing-like panels, and assay comparison studies.
Discussion of Survey Responses

BACKGROUND
In February and March, 2010, the Association of Public Health Laboratories distributed a Training Needs Assessment (TNA) survey to the 94 state and local public health laboratories to assess the testing capabilities, training needs and proficiency testing gaps in Vaccine-Preventable Diseases (VPD) testing at these facilities. The survey also sought to elicit opinions on the value of PHL Centers of Excellence (COEs), including the responding laboratories’ interest in utilizing such a center or, with funding and support, their willingness to become a COE for any of the VPD reference testing addressed in the survey. There were 68 respondents but only 59 of which performed testing of one or more agents of VPDs and completed the survey.

METHODS
The questions were organized by testing topics, as shown in List 1. Organisms addressed in the assessment were listed in the introductory section of the survey so respondents whose laboratory did not test for any of the organisms could stop and return the survey at this point. Skip patterns were incorporated into the survey so that respondents would only have to read and answer questions that were relevant to their laboratory situation.

A pilot test of the survey was sent to five PHLs in the last week of January 2010. This same week, a pre-survey email was sent to the directors of the 94 PHLs stating that they would soon be receiving the survey link via email. They were also advised that completion of the assessment might require input from several individuals in more than one area of the laboratory, including upper management personnel such as the laboratory director.

After the pilot survey responses were reviewed, an edited final version of the survey was made available via Internet link to the directors of the 94 PHLs on February 2, 2010. A printable version of the survey was also sent by email so that the labs could distribute the questions to the appropriate personnel and the responses could be entered into the survey program by one individual once all sections had been completed.

Data from the survey responses were exported from MRInterview into Microsoft Excel for analysis. Data were formatted into tables by question number to facilitate review. Analysis was also done of laboratories’ responses to multiple questions.

DISCUSSION OF SURVEY RESPONSES:
Bacteriology Testing Capability
Culture and speciation by biochemical testing are used by all responding laboratories for testing of at least one of the bacterial VPDs included in this survey. Fifteen (25%) laboratories also perform antimicrobial susceptibility testing for at least one of these bacterial agents. Slide agglutination

List 1
Survey Questions Organization:
1. Introduction
   a. Contact information
2. Bacteriology (4 bacterial agents)
   a. B. pertussis
   b. S. pneumoniae
   c. N. meningitidis
   d. H. influenzae
   e. Proficiency testing
   f. Training
3. Virology (5 viral agents)
   a. Rotavirus
   b. MMRV: Measles, Mumps, Rubella, and Varicella-Zoster Viruses
   c. Training, assay comparison, and proficiency testing
4. General questions; Lab Director level (Bacteriology & Virology)
   a. Training
   b. Centers of Excellence

Table 1
B. pertussis testing capability
(\% of labs, N=59)

<table>
<thead>
<tr>
<th>Method</th>
<th>% of Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>64%</td>
</tr>
<tr>
<td>Biochemical</td>
<td>66%</td>
</tr>
<tr>
<td>DFA</td>
<td>5%</td>
</tr>
<tr>
<td>Seroglutination</td>
<td>2%</td>
</tr>
<tr>
<td>Single-target PCR</td>
<td>0%</td>
</tr>
<tr>
<td>Multi-target PCR</td>
<td>53%</td>
</tr>
<tr>
<td>Antigen capture</td>
<td>25%</td>
</tr>
</tbody>
</table>
serotyping/serogrouping is commonly performed for *N. meningitidis* (85%) and *H. influenzae* (68%). PCR-based methods are used for *B. pertussis* identification by 75% of laboratories, but less than 17% of laboratories perform PCR-based methods for identification and/or serotyping/serogrouping for any of the other bacterial agents included in the survey.

**Bacteriology Testing Capability: Bordetella pertussis**

Culture and speciation by biochemical testing for *B. pertussis* are performed by most of the responding laboratories. Table 1. A majority of laboratories perform culture (88%) and *B. pertussis* speciation by DFA (66%) or biochemical tests (64%). However, only 5% of laboratories reported performing *B. pertussis* speciation by seroglutination. One laboratory reported performing *B. pertussis* speciation by colony morphology only. Not unexpectedly, none of the responding laboratories perform the serotyping test for *B. pertussis*. Three (5%) of the laboratories do not perform testing for *B. pertussis* by any of the methods listed in survey.

Of the responding laboratories, 44 (75%) report performing PCR to test for *B. pertussis*. Single-target PCR is performed by 31 (53%) laboratories and multiplex PCR by 15 (25%), with two (3%) laboratories reporting they do both. Twelve different genes were indicated by the laboratories as targets of their single and multiplex PCR methods. Table 2. Gene target IS481 is used by over 90% of the 44 laboratories doing PCR, followed by BP485 (11%), ptX (7%), ptxA (5%), *B. parapertussis* IS1001 (5%), and a variety of other gene targets utilized by individual laboratories, including: Aprn, 16s, IS1663, porine gene, PTXS1, recA, and BP283. Most (70%) of these laboratories indicated that they are not using a commercial kit for *B. pertussis* PCR. Those which do stated that they use Cepheid (9 laboratories), Roche (three laboratories), and Eragen (one laboratory). While the Roche kit was discontinued prior to the distribution of this survey, these three laboratories were using kits that remained in their stock. Most (78%) of the 59 responding laboratories indicated that they already have sufficient information to properly evaluate assay performance for the commercially available PCR kits.

The survey stated that the FDA and CDC have developed a single serum dilution-based ELISA to detect and quantify anti-pertussis toxin IgG antibodies which has proven to be very useful for diagnosis of pertussis in the late phases of disease or during outbreak response and will provide accurate results even after recent vaccination. Given this information, although 42 (71%) of the responding laboratories stated that this assay would improve their laboratory’s ability to evaluate pertussis-like cough illness, only 18 (31%) of the laboratories stated that they would implement this assay in their laboratories. Nineteen (32%) stated that they would not implement this assay, and 22 (37%) were unsure about implementing this assay, stating a variety of concerns, the most often stated being staffing, funding, and a need to solicit input from epidemiology departments before bringing on new testing. Ten of the 19 laboratories which indicated they would not implement this serological testing stated that they felt this test method is unnecessary due to lack of case definition requirements. In addition, only 17 (29%) of responding laboratories indicated an interest in participating in a comparison study of serology assays for *B. pertussis*. This is in contrast to 33 (56%) of laboratories that indicated an interest in participating in a comparison study of PCR assays for *B. pertussis*.

**Bacteriology Testing Capability: Streptococcus pneumoniae**

A majority (86%) of the 59 responding laboratories are able to perform *S. pneumoniae* culture identification, with 22% also doing antibiotic susceptibility testing. Table 3. Serotyping capability, however, is very rare, with only 2% doing serotyping by slide agglutination, 3% by quellung, and 3% by conventional PCR. No laboratories

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Number of laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS481 Only</td>
<td>26</td>
</tr>
<tr>
<td>IS481 &amp; BP485</td>
<td>4</td>
</tr>
<tr>
<td>IS481 &amp; parapertussis IS1001</td>
<td>2</td>
</tr>
<tr>
<td>IS481 &amp; ptX</td>
<td>1</td>
</tr>
<tr>
<td>IS481, ptX, &amp; BP485</td>
<td>1</td>
</tr>
<tr>
<td>IS481 &amp; ptxA</td>
<td>1</td>
</tr>
<tr>
<td>IS481 &amp; PTXS1</td>
<td>1</td>
</tr>
<tr>
<td>IS481 &amp; BP283</td>
<td>1</td>
</tr>
<tr>
<td>IS481, 16s, &amp; IS1663</td>
<td>1</td>
</tr>
<tr>
<td>IS481 &amp; porine gene</td>
<td>1</td>
</tr>
<tr>
<td>ptX only</td>
<td>1</td>
</tr>
<tr>
<td>Aprn only</td>
<td>1</td>
</tr>
<tr>
<td>ptX, ptxA, &amp; recA</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
</tbody>
</table>
reported using real-time PCR for serotyping of *S. pneumoniae*. Eight (14%) laboratories reported not using any of the serotyping methods included in the survey.

Most (74%) of the 54 laboratories that are not doing serotyping reported that this type of testing is seldom requested; cost (43%) and staffing shortages (31%) were other factors indicated. Nearly half (46%) of these 54 laboratories reported that they refer serotyping to a reference laboratory, mainly due to infrequent need for this testing, with time and cost efficiency also noted as considerations.

Of the five (8%) laboratories doing *S. pneumoniae* serotyping by one of the previously mentioned methods, three (60%) indicated that they are able to test for all serotypes, one (20%) for the PCV7- and PCV13-targeted serotypes as well as the 23 valent-targeted serotypes, and one (20%) for 28 serotypes by the CDC’s PCR procedures. The three laboratories using conventional serotyping all stated that they would consider implementing a PCR-based method as an alternative to serological determination of serotypes.

Training in the application of real-time PCR detection of culture negative *S. pneumoniae*, sterile site specimens, or specialized carriage investigations was reported by 31% of responding laboratories as being helpful for their laboratories. All of these laboratories also indicated that protocols, reagents, and validation and proficiency testing panels would be needed to implement this testing.

Thirty-seven (63%) of the responding laboratories indicated that they would be willing to send surveillance data from pneumococcal disease cases directly to CDC. Most (81%) of these 37 indicated that invasive disease incidence data from cases of isolation of organisms from sterile sites could be shared with CDC. Seven (54%) of thirteen laboratories doing antibiotic susceptibility testing could send these data from disease cases, and four (90%) of five laboratories performing serotyping stated they could send data to CDC regarding serotype distribution from disease cases.

**Bacteriology Testing Capability: *Neisseria meningitidis***

Most of the responding laboratories are performing culture (92%) and biochemical testing (97%) of *N. meningitidis*. Table 4. However, only a few (15%) are performing antimicrobial susceptibility testing. Most (85%) laboratories are also performing slide agglutination serogrouping of *N. meningitidis* for serogroups A, B, C, W135, X, and Y; all of whom use a commercial vendor for their reagents. In contrast, few responding laboratories are using PCR for *N. meningitidis* detection (10%) or serogrouping (14%). Only one (2%) laboratory reported not performing testing for *N. meningitidis* by any of the testing methods included in the survey.

Nearly all the 57 laboratories doing biochemical testing of *N. meningitidis* perform oxidase testing (95%), with more than half also doing NH strips (60%) and CTA sugars (51%). Numerous other methods were stated for biochemical testing as well.

Of nine laboratories that reported performing antimicrobial susceptibility testing for *N. meningitidis*, all (100%) perform the Etest or other gradient strip; four (44%) reported using disk diffusion and one (11%) using broth micro dilution. None of these laboratories reported performing agar dilution or automated testing. Eight
(89%) of these nine laboratories test for penicillin G, ciprofloxacin, ceftriaxone, and rifampin; six (67%) test for azithromycin; three (33%) each for tetracycline/minocycline and TSX (Trimethoprim-Sulfamethoxazole); and one (11%) each for a variety of other antibiotics.

Of six laboratories performing PCR detection of *N. meningitidis*, five (83%) reported using an assay targeting the *ctrA* gene. One (17%) of these six laboratories also targets the *porA* gene. One (17%) laboratory uses a PCR assay that targets the *crgA* gene, and no laboratories use a PCR targeting the *sodC* gene. Of the 53 laboratories that reported not using PCR for detection of *N. meningitidis*, 72% indicated low testing volume and 53% indicated cost as factors for not performing this testing. Inadequate staffing (30%), lack of a commercial product (23%), and no case definition requirements (17%) were other factors reported. Additionally, two (4%) of these 53 laboratories stated that they would be implementing this assay in the near future.

Eight (89%) of the nine laboratories that do not perform slide agglutination serogrouping for *N. meningitidis* indicated their main reason for not doing so was low testing volume. In addition, three (33%) laboratories indicated cost was an issue. None of these laboratories indicated an interest in implementing this testing.

Most (74%) of the 50 laboratories using slide agglutination for serogrouping, as well as five (63%) of the eight laboratories performing PCR-based serogrouping, indicated that they would find it useful to implement a QC program/specimens for this testing. (It should be noted that these eight laboratories also perform slide agglutination serogrouping.) Of the 51 laboratories not performing PCR-based serogrouping, 15 (29%) indicated that implementing this testing would be useful for their laboratories. Nearly all (93%) of these 15 laboratories indicated that they would need training, protocols, reagents, and validation and proficiency testing panels in order to implement this testing. Additionally, 24 (47%) of the 51 laboratories not doing PCR-based serogrouping indicated no interest in implementing this testing. Low testing volume (88%), cost (50%), and inadequate staffing (25%) were reported by these 24 laboratories as the main reasons for not wanting to implement this testing.

**Bacteriology Testing Capability: *Haemophilus influenzae***

Most of the responding laboratories reported performing culture (88%) and biochemical testing (97%) of *H. influenzae*. Table 5. Fewer laboratories are performing ß-lactam (37%) or antimicrobial susceptibility testing (17%). Many laboratories are also performing slide agglutination serotyping of *H. influenzae* for serotypes a-f (68%), as well as three (5%) laboratories that stated they test for serotype “b” only. In contrast, few responding laboratories are using PCR for *H. influenzae* detection (5%) or serotyping (3%). Only two (3%) laboratories reported not performing testing for *H. influenzae* by any of the methods included in the survey.

Nearly all of the 57 laboratories doing biochemical testing of *H. influenzae* perform oxidase testing (88%), with more than half also using NH strips (58%). Few of these laboratories indicated performing Haemophilus ID Quad Plates (23%) or Kilian’s test (porphyrin test) (18%). Numerous other methods were stated for biochemical testing as well.

Of ten laboratories that reported performing antimicrobial susceptibility testing for *H. influenzae*, six (60%) perform disk diffusion, five (50%) perform the Etest or other gradient strip, and one (10%) laboratory reported doing broth micro dilution. None of the laboratories indicated performing agar dilution or automated testing. Eight (80%) of these laboratories test for ampicillin, five (50%) test for ceftriaxone, and one (10%) each for a variety of other antibiotics.

Of the three laboratories performing PCR detection of *H. influenzae*, two (67%) use an assay targeting the *bexA* gene and one (33%) other uses an assay targeting the *frdB* gene. None of these laboratories
reported using a PCR that targets *hpD* or *ompP2*. Of the 56 laboratories that reported not using PCR for detection of *H. influenzae*, 73% indicated low testing volume and 52% indicated cost as factors for not performing this testing. Inadequate staffing (34%), lack of a commercial product (16%), and no case definition requirements (14%) were also cited as factors. Additionally, two (4%) of these laboratories stated that they would be implementing this assay in the near future.

Low testing volume was cited by 15 (79%) of the 19 laboratories not performing this testing as the main reason for not using slide agglutination serotyping for *H. influenzae*; while seven (37%) laboratories indicated cost. Only two (11%) of these laboratories indicated an interest in implementing this testing.

Most (68%) of the 40 laboratories using slide agglutination for serotyping indicated that they would find it useful to implement a QC program/specimens for this testing. One (50%) of the two laboratories performing PCR-based serotyping also indicated that implementing a QC program/specimens would be useful for their laboratory. (It should be noted that these two laboratories using PCR-based serotyping are laboratories that also perform slide agglutination serotyping and PCR detection of *H. influenzae*.) Of the 57 laboratories not doing PCR-based serotyping, 15 (26%) indicated that implementing this testing would be useful for their laboratories. Nearly all of them indicated that they would need validation and proficiency testing panels (100%), protocols (93%), reagents (93%), and training (73%) in order to implement this testing. Additionally, 25 (44%) of the 57 laboratories not doing PCR-based serotyping indicated that they were not interested in implementing this testing. The main reasons indicated by these 25 laboratories for not wanting to implement this testing were low testing volume (84%), cost (40%), and inadequate staffing (24%).

**DISCUSSION OF SURVEY RESPONSES: Virology Testing Capability**

Few (32%) responding laboratories reported testing for rotavirus by any of the methods included in the survey, in contrast to measles, mumps, rubella, and varicella-zoster viruses (MMRV) for which testing is still widely performed by various methods. Serology is performed by more than half of the responding laboratories to test for both IgM and IgG MMRV antibodies, with the exception of IgM for mumps and varicella-zoster. More than half the responding laboratories use viral culture for all MMRV viruses, with the exception of rubella. In contrast, for molecular testing, 58% of responding laboratories reported not performing a non-LRN (Laboratory Response Network) PCR for any of the MMRV viruses. Although 76% of responding laboratories have the LRN assay for varicella-zoster detection, 36% report this as being the only PCR assay used for MMRV detection.

Nearly half (44%) of the responding laboratories indicated that they have insufficient information on assay performance for all the viral VPDs to properly evaluate the commercially available ELISA and PCR kits for the viral VPDs.

**Virology Testing Capability: Rotavirus**

Only 7% of responding laboratories indicated that they perform viral culture for rotavirus. Table 6. Serology is performed by 22% of laboratories, with 15% using the Premier Rotaclone EIA (Meridian Biosciences, Inc.) and one (2%) laboratory each using Pathfinder, Remel Expect EIA, Bio-Rad, and ImmunoCard Stat Rotavirus kits.

Only 10% of responding laboratories indicated they test for rotavirus using PCR. Two (3%) laboratories reported performing RT-PCR and four (5%) laboratories reported using real-time RT-PCR; with one (2%) laboratory doing both. One (2%) of the laboratories performing real-time RT-PCR also indicated they test specimens other than stool, such as CSF from cases of afebrile seizures occurring in association with rotavirus gastroenteritis. Only one (2%) laboratory reported performing rotavirus multiplex PCR-based genotyping to help monitor the effectiveness of rotavirus vaccines.

A majority (68%) of laboratories indicated that they do not test for rotavirus by any of the methods included in the survey. Of the 46 laboratories not
performing serology, only 17% indicated an interest in implementing EIA testing for rotavirus. However, 16 (30%) of the 54 laboratories not performing RT-PCR indicated an interest in implementing this testing to monitor vaccine effectiveness. Lastly, nine (16%) of the 58 laboratories not performing genotyping indicated an interest in implementing this testing.

**Virology Testing Capability: Measles, Mumps, Rubella, and Varicella-Zoster Viruses (MMRV)**

A majority of responding laboratories reported performing serology testing for MMRV, with only 17% of laboratories not using IgM or IgG serology for any of the viruses included in the survey. Measles IgM and IgG are performed by 59% and 63% of responding laboratories respectively; mumps IgM and IgG by 34% and 51%; rubella IgM and IgG by 60% and 68%; and varicella-zoster IgM and IgG by 19% and 51%. Tables 7-10.

Many different commercial, in-house, and CDC-developed serology assays were reported as being used by the responding laboratories. No specific kits were used by a majority of the laboratories for any of the serological testing types included in the survey. However, usually one assay was used more often than any others. For measles IgM testing, the Millipore (Chemicon, Light Diagnostics) kit was most commonly used, by 11 (31%) of the 35 laboratories performing this testing. For mumps IgM, the BluePoint Biosciences (Microimmune) kit was most commonly used, by 25% of 20 laboratories performing this testing. For all of the other serology assays included in the survey, the Inverness (Wampole) kits were the most commonly reported kits being used: the percentages ranging from 39% of 36 laboratories for measles IgG testing to 22% of 27 laboratories for varicella-zoster IgG testing.

Although IgM testing for mumps and varicella-zoster are less commonly performed than the other serology tests included in the survey, PCR testing for mumps and varicella-zoster are reported as being used significantly more than for measles and rubella. Whereas only 14% of laboratories perform measles PCR and 5% perform rubella PCR, 41% perform mumps PCR and 76% perform varicella-zoster PCR. It should also be noted, however, that of the 45 laboratories doing varicella-zoster PCR, all reported using the LRN assay. Six of these laboratories (10% of the responding laboratories) also reported performing a non-LRN PCR assay for varicella-zoster. In fact, 36% of laboratories perform the LRN PCR assay for varicella-zoster to the exclusion of any other PCR for MMRV. In addition, 22% of laboratories reported not performing PCR testing for any of the MMRV viruses. Nearly all the laboratories that are performing PCR testing for MMRV are using either CDC or in-house developed assays.

For the laboratories not performing PCR testing for MMRV, a majority indicated that they would be interested in implementing measles (71%) and rubella (55%) PCR testing. Slightly less than half indicated interest in implementing mumps (49%) and varicella-zoster (42%) PCR testing. Also, 44% of all responding laboratories indicated that they do not feel they have sufficient information on assay performance to properly evaluate the commercially available ELISA and PCR kits for rotavirus and MMRV viruses. Over half (54%) of the responding laboratories indicated that they would be willing to participate in comparison studies of measles and mumps EIAs to determine how well kits perform in terms of sensitivity and specificity for detecting IgM and IgG. When asked specifically what ELISA and/or PCR
assays they would like to have more information on from a comparison study, laboratories generally indicated PCR assays for MMRV viruses.

Viral culture is reported as being performed by most laboratories for varicella-zoster (68%), mumps (62%), and measles (53%), but is more rarely used for rubella (19%) testing. Rhesus monkey kidney cells (RMK) were reported as being used far more than any other cell lines for viral culture of measles (80% of 31 laboratories) and mumps (83% of 36 laboratories). However, for these two viruses there were many other cell lines also reported as being used by the laboratories, often in conjunction with RMK cells; most notably Vero, Hep-2, MRC-5, and A549. For varicella-zoster viral culture, MRC-5 was the most commonly reported cell line used (63% of 40 laboratories), followed by RMK and A549. The 19% of responding laboratories performing rubella viral culture did not report an overwhelmingly higher use of any specific cell line. Nearly one-third (29%) of responding laboratories did not report performing viral culture testing for any of the MMRV viruses.

Of the responding laboratories, 17% did not report performing testing for rubella, 15% for measles, 15% for mumps, and 5% for varicella-zoster by any of the testing methods included in this survey (serology, viral culture, and PCR). An additional 12% of laboratories reported the LRN procedure as the only method being used for varicella-zoster testing.

DISCUSSION OF SURVEY RESPONSES: Proficiency Testing

As seen in the responses earlier in this report, a much larger number of laboratories are performing testing for the bacterial agents included in this survey, with a majority of laboratories doing culture for these organisms, than the number of laboratories doing testing for any of the viral agents. As a reflection of this, the number of laboratories that report they could share bacterial cultures as part of a PT program was much higher than the number of laboratories that could share viral cultures. Similarly, the number of laboratories that report a need for bacterial cultures for proficiency testing (PT) needs was lower than the number of laboratories with a need for viral cultures. In general, when asked what APHL could do to further assist laboratories with their proficiency testing quality assurance and quality improvement programs for the bacterial and viral VDPs included in this survey, the responding laboratories most often asked for PT panels or programs to be set up. In addition, the responding laboratories asked for assistance with coordinated specimen exchange programs and for further information on existing PT panels available for the VPDs. Most laboratories reported that they are not currently participating in a sample sharing program with another laboratory for QA or PT testing purposes: ten (16%) laboratories share samples for *B. pertussis* testing, eight (14%) for mumps, six (10%) for measles, five (8%) each for rubella and varicella-zoster, and one (2%) for *H. influenzae*.

In the next sections, the survey asked the respondents if they felt their laboratory would benefit, based on their currently available testing, from implementing new proficiency testing panels for the bacterial and viral testing methods included in the survey. These summarized responses are from the laboratories currently performing each type of testing.

**Bacteriology Proficiency Testing**

Of 52 responding laboratories doing *B. pertussis* culture, 34 (65%) indicated a need for further PT panels. Table 11. Also, 36 (82%) of 44 laboratories doing PCR for *B. pertussis* indicated a need for PT panels for single-target and multiplex PCR.

The laboratories also indicated a need for *N. meningitidis* and *H. influenzae* PT panels for the testing they are already performing: 34 (68%) of 50 laboratories need panels for *N. meningitidis* slide agglutination serogrouping, and 29 (54%) of 54 laboratories for *N. meningitidis* culture. Also, 28 (70%) of 40 laboratories need panels for *H. influenzae* serotyping, and 26 (50%) of 52 laboratories for *H. influenzae* culture. Nearly three-quarters of laboratories doing slide agglutination testing for these two organisms indicated that...
implementing a QC program/specimens for slide agglutination serogrouping/serotyping would be useful for their laboratory. Also, nearly one-third of responding laboratories indicated a need for positive specimens to validate existing and new assays: 32% for *N. meningitidis* and 30% for *H. influenzae*.

Nineteen (37%) of 51 laboratories doing *S. pneumoniae* culture indicated a need for PT panels for this testing. By contrast, there was little need indicated for PT panels for the other testing methods included in the survey, reflected by a dearth in the number of laboratories actually performing the testing.

Most of the laboratories that are using slide agglutination serogrouping/serotyping for the bacterial agents included in the survey indicated that they would or might implement the PCR-based typing assays: one (100%) of one laboratory for Pneumococcus, 27 (53%) of 51 laboratories for Meningococcus, and 32 (57%) of 57 laboratories for *H. influenzae*. PT panels for these organisms would help laboratories to set up and validate the assays in their laboratories.

### Virology Proficiency Testing

Since 68% of laboratories do not do rotavirus testing of any type, there were few laboratories that indicated they would benefit from implementing PT panels for rotavirus testing: One (8%) of 13 laboratories doing serology, and five (83%) of six laboratories doing PCR. **Table 12.** In contrast, many laboratories perform viral serology for measles, mumps, rubella, and varicella-zoster viruses and there were a high number of laboratories indicating a need for these panels: 25 (60%) of 42 laboratories for measles, 24 (51%) of 47 laboratories for rubella, 20 (56%) of 36 laboratories for mumps, and 15 (48%) of 31 laboratories for varicella-zoster. For molecular testing, 19 (79%) of 24 laboratories performing mumps PCR indicated a need for this testing. However, there was little need indicated by the responding laboratories for measles, rubella, or varicella-zoster PCR panels; the only exception to this being the 23 (51%) of 45 laboratories doing varicella-zoster PCR that indicated a need for PT panels for this testing.

More than half of the responding laboratories reported that they are already performing serology testing for measles, mumps, rubella, and varicella-zoster viruses, and about half of these laboratories also indicated that they would benefit from implementing new proficiency testing panels for these testing methods. Over half of the responding laboratories also indicated a need for positive viral specimens to validate existing and new assays: 56% for measles, 53% for mumps, 49% for rubella, and 58% for varicella-zoster. However, less than 20% of
Table 12
Need For New Virology Proficiency Testing Panels
(# of labs, N=59)

<table>
<thead>
<tr>
<th>Test</th>
<th>Labs currently doing testing</th>
<th>Labs not currently doing testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles Serology</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Rubella Serology</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Varicella PCR, any (incl. LRN)</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Mumps Serology</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Mumps PCR</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>Varicella Serology</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Measles PCR</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Varicella PCR, non-LRN</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Rotavirus PCR</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Rubella PCR</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Rotavirus Serology</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 13
Hands-on Training Interest: Bacteriology
(# of labs, N=59)

<table>
<thead>
<tr>
<th>Test</th>
<th>Labs doing testing</th>
<th>Labs not doing testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. pertussis multiplex PCR</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>N. mening PCR-based serogrouping</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>H. influ PCR-based serotyping</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>H. influ PCR (general)</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>N. mening PCR (general)</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>S. pneumo PCR serotyping</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>H. influ slide aggl: QC/troubleshooting</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>N. mening slide aggl: QC/troubleshooting</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>H. influ slide aggl: basic</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>N. mening slide aggl: basic</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>S. pneumo slide aggl</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
laboratories indicated that they have cultures which they could share as part of a PT program or that they participate in a sample sharing program with another laboratory for QA/PT purposes.

A large number of the laboratories that are not doing PCR for detection of measles, mumps, rubella, and varicella-zoster viruses indicated they would be interested in implementing the assays in their laboratories. PT panels for these organisms are essential in order for these laboratories to set up and validate the assays.

**DISCUSSION OF SURVEY RESPONSES: Training**

Responding laboratories indicated that training for VPD testing is obtained from a variety of sources, including: NLTN (APHL) teleconferences and/or hands-on laboratory workshops (90%), CDC published protocols (86%), in-house training by other staff (80%), use of CLSI guidance documents (76%), instrument manufacturers (61%), other public health laboratories (31%), other professional organizations (10%), and/or clinical laboratories (7%).

Responding laboratories also provide training or outreach activities related to VPD testing to clinical laboratories, including: when expertise is requested (53%), providing teleconferences (19%), information on their websites (17%), newsletters (15%), panels of challenge organisms (14%), reference materials (such as CLSI documents) (14%), in-person lectures (10%), and/or hands-on laboratory workshops (9%).

Approximately one-third to one-half of all responding laboratories indicated that they would be interested in participating in teleconferences and self-study web-based trainings for all the testing methods included in the survey, including PCR for testing of rotavirus, measles, mumps, rubella, and varicella-zoster viruses, and B. pertussis; and slide agglutination and PCR serogrouping/serotyping of S. pneumoniae, N. meningitidis, and H. influenzae. (Range of 32-56% for teleconferences and 24-48% for web-based trainings.) Tables 13 & 14.

Responding laboratories indicated interest in each of the methods included in the survey for hands-on laboratory training. Table 15. Many of the laboratories which indicated a training interest were those laboratories that were not currently performing testing for these same methods. For instance, 25 laboratories indicated an interest in B. pertussis multiplex PCR training, of which 20 also reported not currently performing this testing; the assumption being that they would have interest in implementing the test in their laboratories. Also, for bacteriology, 21 laboratories indicated interest in training for N. meningitidis PCR-based serogrouping, 20 for H. influenzae PCR-based serotyping, and 15 for S. pneumoniae PCR serotyping; with 13 of these being laboratories interested in all three types of training and not currently performing this testing. There were similar numbers of laboratories indicating interest in training for PCR identification of N. meningitidis and H. influenzae, but slightly less interest for training in slide agglutination for these two organisms.

For virology, 24 laboratories indicated an interest in hands-on training in PCR for measles and mumps, 23 for rubella, and 22 for varicella-zoster. Nineteen of these responses were laboratories that indicated interest in hands-on training in PCR for all four of these viruses. Table 16. By contrast, there was less interest in testing methods training for rotavirus serology, PCR, and genotyping.

**DISCUSSION OF SURVEY RESPONSES: Centers of Excellence**
This Training Needs Assessment survey served as a tool to collect preliminary information to assess whether public health laboratories would be willing to serve as, or utilize the services of, regional Centers of Excellence (COEs) to test for those pathogens for which they do not have the capability to detect or have a low testing demand. The assessment also served to identify potential barriers in the utilization of COEs.

Thirty (51%) of the responding laboratories indicated that they would utilize a regional COE for VPD reference testing for at least one of the pathogens included in this survey. There was not a significant difference between the percentage of SPHLs (47%) and LPHLs (60%) who responded in the affirmative. Of the

<table>
<thead>
<tr>
<th>Table 15</th>
<th>Interest in Utilizing COEs for reference testing</th>
<th>(# of labs, N=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertussis PCR rapid diagnostics</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Pneumococcus serotyping (slide agglutination and/or PCR)</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Meningococcus serogrouping</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>H. influenzae serotyping</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Measles</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Mumps</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Rubella</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>Varicella</td>
<td>9</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 16</th>
<th>Willing to be a COE testing site</th>
<th>(# of labs, N=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertussis PCR</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>Pneumococcus serotyping (slide agglutination and/or PCR)</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Meningococcus serogrouping</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>H. influenzae serotyping</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>Measles</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Mumps</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Rubella</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Varicella</td>
<td>22</td>
<td>17</td>
</tr>
</tbody>
</table>
remaining laboratories, 26 (44%) indicated that they are uncertain as to what capacity (if any) they would utilize a regional COE, while only three laboratories (5%) indicated they would not be interested in using the services of COEs.

Twenty-one (70%) of the 30 laboratories interested in COEs indicated that they would utilize the service of COEs for VPD reference testing of at least two of the pathogens included in the survey. The primary reasons indicated as being barriers to the utilization of COEs were the cost burden of shipping specimens (73%) and the cost of reagents and controls (63%). Other barriers indicated by the responding laboratories included issues surrounding patient confidentiality (20%), reporting to epidemiology departments (20%), and legal restrictions to sharing specimens across states (14%).

If provided with sufficient funding and support, such as reagents and proficiency testing specimens, 54% (23 SPHLs & 9 LPHLs) of the responding laboratories indicated that they could serve as a regional COE for VPD reference testing for at least one of the pathogens included in the survey. Table 18 shows the percentages of laboratories willing to provide testing services for the types of testing included in the survey. If sufficiently supported with funding, reagents, and proficiency testing specimens, more than half of the laboratories that indicated they could serve as regional COEs also indicated that they could offer testing services for at least seven of the pathogens included in the survey; and 10% of laboratories indicated that they could test for all of them. Of the remaining laboratories, 25% were uncertain as to what testing capacity (if any) they would be able to offer, while 20% indicated that they would not be interested in serving as a regional COE.

A major barrier to becoming a COE is the availability of sufficient staff, as indicated by 83% of responding laboratories. Other potential barriers to becoming COEs include the lack of necessary instrumentation (36%) and the laboratories’ mandate/mission (25%). Only 7% of responding laboratories indicated that they do not perceive any barriers to becoming a COE.
RECOMMENDATIONS

BACTERIAL AND VIRAL VPD TESTING CAPABILITY

Assay comparison studies
Responding laboratories reported using a number of commercial ELISAs and many of the respondents (44%) reported they do not have sufficient information to evaluate these kits, indicating the usefulness of instituting assay comparison studies. Mumps and measles serology kits are widely used for disease diagnosis and can provide vital information during outbreaks. Many laboratories also report using rubella serology kits, but the incidence of this disease and importance for rapid diagnostics are much lower than for measles and mumps.

Based on the survey responses, it is recommended that assay comparison studies be performed on the commercially available ELISA kits for measles and mumps serology.

BACTERIAL AND VIRAL VPD PROFICIENCY TESTING

Pilot Proficiency Testing Panels
Several possible pilot proficiency testing (PT) panel options have been proposed based on the needs identified in the previous section. List 4. The first four options could be utilized both by laboratories doing testing, to assess their current testing methods, while also being used by laboratories not yet doing testing, to perform assay validation studies for the implementation of new tests in their laboratory.

A pilot PT panel for pertussis could potentially be used for both culture and PCR testing. Also, the responding laboratories indicated previously in the survey that they are using various combinations of gene targets for identification of pertussis by their in-house PCR assays. A panel could be used to help assess how well these different gene targets compare in their abilities to identify and distinguish between Bordetella spp. organisms. These results could be used to analyze issues behind different PCR methods, different instruments utilized, and standardization of protocols. About one-third (34%) of the responding laboratories indicated a need for positive B. pertussis specimens to validate existing and new assays, and 28% requested PT specimens/program or a specimen exchange program, more than for any of the other bacterial agents included in the survey.

Based on the survey responses, it is recommended that pilot proficiency testing panel exercises be initiated with the state and local PHLs for one or more of the options stated in List 4.

List 4
Pilot Proficiency Testing Panel Options:
1. Bordetella spp. panel for culture and PCR identification testing
2. N. meningitidis panel for culture, PCR identification, and slide agglutination and PCR-based serogrouping
3. H. influenzae panel for culture, PCR identification, and slide agglutination and PCR-based serogrouping
4. Viral serology panel for measles, mumps, rubella, and varicella-zoster
5. Validation panels for laboratories to implement PCR-based serogrouping/serotyping for Pneumococcus, Meningococcus, and/or H. influenzae
6. Validation panel for laboratories to implement PCR for measles, mumps, rubella, and/or varicella-zoster

BACTERIAL AND VIRAL TRAINING

Teleconferences
The highest interests indicated in the survey for teleconference trainings were for:

• QC/ troubleshooting/ difficult case studies for meningococcus and H. influenzae slide agglutination
• Multiplex PCR for pertussis
• PCR for measles, mumps, rubella, and varicella-zoster viruses
Based on these responses, the recommendation is that teleconferences on QC/ troubleshooting/ difficult case studies for meningococcus and *H. influenzae* slide agglutination, pertussis multiplex PCR, and PCR for measles, mumps, rubella, and varicella-zoster viruses be developed and presented to the PHLs.

**Web-based trainings**

The highest interests indicated in the survey for web-based self-study teleconference trainings were the same as for teleconferences:

- QC/ troubleshooting/ difficult case studies for meningococcus and *H. influenzae* slide agglutination
- Multiplex PCR for pertussis
- PCR for measles, mumps, rubella, and varicella-zoster viruses

The survey responses also indicated that trainings on general topics, such as basic method validation for PCR assays, are needed by laboratories to teach staff on how to implement new tests in their laboratories.

Based on the survey responses, the recommendation is that web-based trainings on these basic techniques be developed and presented to the PHLs to assist in implementation of new tests.

**Hands-on Laboratory Workshops**

Based on the responses to the hands-on training questions, the training topics that could be taught in a 3-5 day course have been grouped into the scenarios identified in Lists 5 and 6.

**Bacterial VPDs**

- Pertussis multiplex PCR
- PCR-based serotyping/typing
- Slide agglutination

**Viral VPDs**

- PCR for measles, mumps, rubella, and varicella-zoster viruses
- Rotavirus RT-PCR and genotyping

Several possible training scenarios based on these groupings have been proposed, see Lists 5 and 6. Course topics would need to include protocols, assay validation, best practices, and vendor options as a majority of the laboratories that expressed interest in the proposed trainings are laboratories that are not currently doing testing.

Course content for pertussis multiplex PCR training should discuss the issues behind different PCR methods and instruments utilized, including standardization of protocols, as the laboratories that are performing pertussis PCR use many different gene targets for this testing. About half of the laboratories that indicated an interest in training for PCR-based serogrouping/typing for meningococcus, *H. influenzae*, and/or pneumococcus, also indicated elsewhere in the survey that they would like to implement these assays. Such training, therefore, would be beneficial in assisting these laboratories to implement these tests. A majority (59%) of the laboratories that expressed interest in meningococcus and *H. influenzae* hands-on training in slide agglutination serotyping/serogrouping for QC/troubleshooting/difficult case studies are laboratories that are already performing these tests and that would like to implement QC programs/specimens for these assays. Therefore, a training course would need to include a focus on QC, specimen exchanges/sample sharing programs, assay troubleshooting, and difficult case studies.
Many (38-78%) of the laboratories expressing an interest in PCR training for measles, mumps, rubella, and varicella-zoster viruses are laboratories that are not currently doing testing, but that expressed an interest in implementing these assays elsewhere in the survey. For mumps PCR training, approximately one-third (38%) of the laboratories that expressed an interest are laboratories that currently do the testing; another 38% are those that expressed an interest in implementing this assay. A majority (68%) of the 22 laboratories that expressed an interest in varicella-zoster PCR training are the laboratories that currently have the LRN-PCR method. Half (50%) of these 22 laboratories (regardless of LRN capabilities) expressed an interest in implementing this testing. Almost all (93-100%) of the laboratories that expressed an interest in hands-on training for rotavirus RT-PCR or genotyping are laboratories that are not currently performing these types of molecular testing for rotavirus.

The recommendation is that the CDC review these possible hands-on training scenarios as potential courses for implementation.

**CONCLUSION**

Discussion of the recommendations in this report with members of the VPD project Steering Committee has led to the decision to pursue the following initiatives:

**Hands-on Laboratory Workshops**
- Rotavirus genotyping and sequencing
- Bacterial VPDs: molecular methods (PCR for identification and typing)

**Pilot Proficiency Testing panels**
- Pertussis PCR
- Viral serology

**Assay comparison study**
- Measles IgM ELISA

The data gathered in this survey regarding laboratories’ perception of the utilization of COEs will be used to further analyze the feasibility of establishing regional testing centers for select VPD testing in the future.