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International Circumpolar Surveillance Interlaboratory Quality Control Program for Serotyping *Haemophilus influenzae* and Serogrouping *Neisseria meningitidis*, 2005 to 2009

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The International Circumpolar Surveillance (ICS) program was initiated in 1999 to conduct population-based surveillance for invasive pneumococcal disease in select regions of the Arctic. The program was expanded to include the surveillance of invasive diseases caused by *Neisseria meningitidis* and *Haemophilus influenzae*. An interlaboratory quality control (QC) program to monitor laboratory proficiencies in the serogrouping of *N. meningitidis* and serotyping of *H. influenzae* strains was codeveloped by the Arctic Investigations Program (Anchorage, AK) and the Public Health Agency of Canada National Microbiology Laboratory (Winnipeg, Manitoba, Canada) and introduced into the ICS program in 2005. Other participating laboratories included the Provincial Laboratory for Public Health (Edmonton, Alberta, Canada), Laboratoire Santé Publique du Québec (Sainte-Anne-de-Bellevue, Québec, Canada), and Statens Serum Institut (Copenhagen, Denmark). From 2005 through 2009, 50 isolates (24 *N. meningitidis* and 26 *H. influenzae* isolates) were distributed among the five participating laboratories. The overall serogroup concordance for *N. meningitidis* strains was 92.3% (96/104), without including three isolates that were found to express both serogroup Y and W135 specificities. Concordant results were obtained for serogroups A, B, C, and Y among all laboratories. Discrepancies were observed most frequently for serogroups W135, X, Z, and 29E. The overall serotype concordance for *H. influenzae* was 98% (125/127 attempts). The two discrepant results involved a serotype c strain and a serotype e strain, and in both cases, the serotypeable *H. influenzae* isolates were misidentified as being nontypeable. These data demonstrate a high degree of concordance for serogroup and serotype determinations of *N. meningitidis* and *H. influenzae* isolates, respectively, among the five laboratories participating in this quality control program.

The International Circumpolar Surveillance (ICS) program was established to enhance the surveillance of infectious diseases of special interest to circumpolar regions (28, 42). This program initially focused on the surveillance of invasive pneumococcal disease (IPD) in the U.S. Arctic (Alaska) and northern Canada and began in 1999 (30). Soon thereafter, other countries joined (Greenland, Iceland, Norway, Finland, and Sweden) (28), and the program has since expanded to cover the surveillance of other invasive bacterial diseases caused by *Neisseria meningitidis*, *Haemophilus influenzae*, and group A and B streptococci (29). The surveillance of invasive bacterial diseases in the circumpolar regions has been developed under the auspices of the Arctic Council, a ministerial forum of governments from countries in geographical proximity to the Arctic. The Invasive Bacterial Disease Working Group (IBDWG) within the ICS program is made up of both laboratory scientists and clinical epidemiologists working in population/public health. The IBDWG has worked to form a laboratory network throughout the Arctic region with standardized data collection for the long-term monitoring of invasive bacterial diseases, with the ultimate aim of the prevention and control of invasive bacterial diseases.

Essential for the success of any international surveillance program is the need to be able to compare data, which are often generated by using different testing methodologies. In 1999, with the formation of the ICS program for the surveillance of IPD, an interlaboratory quality control (QC) program was introduced. This QC program served as an external proficiency testing mechanism for the serotyping and antibiotic susceptibility testing of *Streptococcus pneumoniae* and provided a means for the monitoring of test results and standard testing procedures across all participating laboratories (30). A similar program that monitors laboratory proficiencies in the serogrouping of *N. meningitidis* and the serotyping of *H. influenzae* isolates was codeveloped in 2004 by the Centers for Disease Control and Prevention Arctic Investigation Program (AIP) (Alaska) and the Public Health Agency of Canada National Microbiology Laboratory (NML) (Winnipeg, Canada). The other laboratories that participate in this QC program include the Provincial Laboratory for Public Health (Edmonton, Alberta, Canada), Laboratoire Santé Publique du Québec (Sainte-Anne-de-Bellevue, Québec, Canada), and Statens Serum Institut (Copenhagen, Denmark).

The introduction of effective conjugated vaccines against subpopulations of *H. influenzae* (serotype b) and *N. meningitidis* (se-

Received 13 July 2011 Returned for modification 23 September 2011
Accepted 7 December 2011
Published ahead of print 14 December 2011
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doi:10.1128/JCM.05084-11

TABLE 1 Methods and reagents used for serotyping of *Haemophilus influenza* and serogrouping of *Neisseria meningitidis*

	Serotyping of *H. influenzae*		Serogrouping of *N. meningitidis*	
Laboratory	Method	Source, reagent, or reference(s)	Method	Source, reagent, and/or reference(s)
1	Slide agglutination	Difco Laboratories	Real-time PCR[a]	27
2	Slide agglutination	Remel (Thermo Fisher)	Slide agglutination	Antisera provided by the National Microbiology Laboratory; 8, 9, 19, 36
			PCR[b]	
3	PCR	15, 18	Slide agglutination	Antisera provided by the National Microbiology Laboratory
4	Slide agglutination	Difco Laboratories	Latex agglutination	Slidex (Pasteur-Merieux), Wellcogen (Remel), Pastorex meningitis kit (Bio-Rad Laboratories)
			Coagglutination	In-house group-specific rabbit antisera; 2
			PCR[c]	8, 9, 36
5	Slide agglutination	Difco Laboratories	Slide agglutination	In-house-produced rabbit antisera; 3
	PCR	15	PCR[d]	5, 8, 9, 36

[a] PCR targeting the *sacB* gene of serogroup A, the *siaD* genes of serogroups B and C, the *synF* gene of serogroup Y, the *synG* gene of serogroup W135, and the *xcbB* gene of serogroup X.
[b] PCR targeting the *sacB* and *mynC* genes of serogroup A, the *siaD* genes of serogroups B and C, the *synF* gene of serogroup Y, and the *synG* gene of serogroup W135.
[c] PCR targeting the *sacB* and *mynC* genes of serogroup A, the *siaD* genes of serogroups B and C, the *synF* gene of serogroup Y, and the *synG* gene of serogroup W135.
[d] PCR targeting the *sacB* gene of serogroup A, the *siaD* genes of serogroups B and C, the *synF* gene of serogroup Y, the *synG* gene of serogroup W135, and unique regions of the *ctrA* genes specific for serogroups 29E, X, and Z.

rogroups A, C, W135, and Y) has led to substantial changes in the epidemiologies of these invasive bacterial diseases. For example, in countries with childhood immunization programs for *H. influenzae* serotype b (Hib), invasive Hib disease has been largely controlled, and the contention that Hib may be eliminated has also been discussed (4). Subsequent to the introduction of Hib conjugate vaccines, non-serotype b *H. influenzae* has now become the major cause of invasive *H. influenzae* disease (1, 10, 11, 14, 24, 38). With the increasing use of vaccines to control invasive meningococcal disease (IMD) caused by serogroups A, C, Y, and W-135 (12, 26) and the recent development and imminent licensure of a serogroup B vaccine (33), a larger proportion of the remaining IMD in the future will be caused by organisms with rare serogroups. As the total number of cases decreases as a result of vaccine use, laboratory staff will have less experience in routine test procedures for the serogrouping and serotyping of these bacteria. At the same time, the chances of encountering less common serotypes and serogroups are increasing. With this change in the characteristics of the predominant organisms coupled with a lack of adequate experience, the serotyping and serogrouping of bacteria can become challenging tasks in the routine clinical microbiology laboratory. Also, discrepancies in the identification of serogroups of *N. meningitidis* and serotypes of *H. influenzae* have been reported in the literature in the last decade (22, 27). Finally, newer laboratory methods for the serogroup and serotype identifications of these pathogens have been introduced in the last 2 decades (5, 7–9, 15, 18, 23, 25, 27, 32, 34, 36). All these developments point toward the importance of the quality control of laboratory procedures used to monitor these invasive bacterial pathogens, especially when vaccine usage and bacterial evolution are occurring in parallel.

Here we report the development of an interlaboratory quality control program for the serogrouping of *N. meningitidis* and serotyping of *H. influenzae* strains and data collected from the first 5 years of this program (2005 to 2009).

MATERIALS AND METHODS

Distribution of isolates. From 2005 to 2009, panels of selected *N. meningitidis* and *H. influenzae* isolates (5 each) were distributed among the following laboratories two times per year (with the exception of 2008, when the two panels were combined, with only one shipment of 10 isolates): the Centers for Disease Control and Prevention Arctic Investigation Program (AIP), the Public Health Agency of Canada National Microbiology Laboratory (NML), the Provincial Laboratory for Public Health (Edmonton, Alberta, Canada), the Laboratoire Santé Publique du Québec (Sainte-Anne-de-Bellevue, Québec, Canada), and the Statens Serum Institut (Copenhagen, Denmark). The distribution dates were agreed upon in advance. Each panel consisted of either two isolates of *H. influenzae* and three isolates of *N. meningitidis* or two isolates of *N. meningitidis* and three isolates of *H. influenzae*. *H. influenzae* isolates were selected to represent either a known serotype or a nonencapsulated or nonserotypeable strain (see Table 2). *N. meningitidis* isolates were selected to represent either a known serogroup or a nongroupable or nonserogroupable strain (see Table 3). The NML distributed isolates on chocolate agar slants. The AIP distributed lyophilized isolates prepared in skim milk. All isolates were shipped according to International Air Transportation Association (IATA) regulations.

Serotyping of *H. influenzae*. The most commonly used methods for the serotyping of *H. influenzae* included either slide agglutination using commercially available antisera (Difco Laboratories, Detroit, MI, and Remel Europe Ltd., Dartford, United Kingdom) and PCR capsule typing based on either a procedure described previously by Falla et al. (15) or a multiplex PCR approach described previously by Gonin et al. (18). Some laboratories employed more than one approach for serotype identification (Table 1).

Serogrouping of *N. meningitidis*. For *N. meningitidis*, the identification of the serogroup was accomplished by slide agglutination using in-house-prepared rabbit antisera (3), or in one laboratory, a variety of commercial latex particle agglutination test kits for serogroups A, B, and C (Slidex [bioMérieux Clinical Diagnostics, France], Pastorex meningitidis kit [Bio-Rad Laboratories], and Wellcogen [Remel]) were used together with staphylococcal coagglutination with in-house-produced rabbit antisera for serogroups W135, X, Y, Z, and 29E (2). Other methods included PCR for the identification of serogroups A, B, C, Y, W135, X, Z, and 29E

TABLE 2 Serotype results for the *H. influenzae* quality control isolates of the International Circumpolar Surveillance program, 2005 to 2009

Yr	No. of isolates	Serotypes (no. of isolates)[b]	% concordance (no. of concordant results/ total no. of tests)
2005	5	a, b, c, e, f	92 (23/25)[a]
2006	6	a, b, c, d, e, f	100 (27/27)
2007	5	a, b, d, NT (2)	100 (25/25)
2008	5	a, b, e, f, NT	100 (25/25)
2009	5	a, b, e, f, NT	100 (25/25)

[a] A serotype c isolate and a serotype e isolate were reported as being nontypeable.
[b] NT, nontypeable.

TABLE 3 Serogroup results for the *N. meningitidis* quality control isolates of the International Circumpolar Surveillance program, 2005 to 2009

Yr	No. of isolates	Serogroups (no. of isolates)	% concordance (no. of concordant results/ total no. of tests)[a]
2005	5	A, B, C, Y, W135	100 (25/25)
2006[b]	4	B, W135, X, Y/W135	85.7 (12/14)[c]
2007	5	C, X, Z (2), 29E	80 (20/25)[d]
2008	5	B (2), W135, Y/W135 (2)	100 (15/15)[a]
2009	5	B, C, Y, W135, 29E	96 (24/25)[e]

[a] Three isolates appeared to express antigenic specificities of both serogroups Y and W135 and were excluded for the calculation of concordance.
[b] One laboratory did not participate in the autumn panel.
[c] Involved one laboratory that identified a serogroup W135 organism as a serogroup X organism and that identified a serogroup X organism as a W135 organism, possibly due to a mix-up of the specimens.
[d] One laboratory failed to identify the serogroup Z organism twice (spring and autumn panels), and another laboratory failed to identify it once; both laboratories were employing the PCR method and reported these organisms as being nongroupable. Another laboratory using real-time PCR misidentified a serogroup 29E organism as a serogroup Y organism. One laboratory, also using PCR, did not identify the serogroup 29E strain and reported it as being nongroupable.
[e] One laboratory using PCR identified the serogroup 29E organism as being nongroupable.

(5, 8, 9, 36) or real-time PCR (27). Some laboratories employed more than one method to determine the serogroup nature of *N. meningitidis* strains (Table 1).

Reporting. A standardized report form to ensure consistent data collection was sent by the distributing laboratory with each QC panel. The information collected on the report form included the test method used and a table for reporting each serotype/serogroup. The report form was completed by each participating laboratory and then returned to the distributing laboratory within 6 weeks. The distributing laboratory was responsible for compiling a summary report of the results, and if specific problems were identified, a discussion was offered by the distributing laboratory to assist with problem solving.

Ownership of isolates. The QC isolates are considered the property of the province, state, or country from which they were distributed. The isolates could be retained for internal reference use but were not shared with other laboratories or used for research purposes by the receiving laboratories without written consent from the distributing laboratory.

RESULTS

Isolates distributed for quality control. During the first 5 years (2005 to 2009) of this quality control program, a total of 50 organisms (26 *H. influenzae* and 24 *N. meningitidis* isolates) were distributed among the five participating laboratories. The serotype and serogroup characteristics of the QC organisms are described in Table 1. The only deviations in the distribution of isolates from the developed protocol were as follows: (i) in 2008, all 10 QC organisms were sent out in one panel instead of two due to some logistic difficulties, and (ii) one laboratory did not participate in the testing of the second panel in 2006.

Serotyping of *H. influenzae*. Of the 127 attempts carried out by the five participating laboratories to determine the serotypes of 26 *H. influenzae* isolates, 125 tests gave the correct serotype result, for an overall concordance of 98% (125/127 attempts). Five serotype a and five serotype b isolates were correctly identified by all laboratories throughout the 5 years of this program. Concordant serotyping results were also obtained among all laboratories for four isolates of serotype f, 4 isolates that were nontypeable, 2 isolates of serotype d, and one isolate each of serotype c and serotype e. Discrepancies between the expected serotype and the reported results were noted in two instances (Table 2). The two discrepant results involved a serotype c strain and a serotype e strain distributed in two separate panels. In both cases, the serotypeable *H. influenzae* strains were identified as being nontypeable; in one laboratory, this was done by a bacterial agglutination test, and in the other laboratory, this was determined by multiplex PCR.

Serogrouping of *N. meningitidis*. A total of 24 *N. meningitidis* isolates were distributed to the five participating laboratories for serogroup determinations. Three isolates were subsequently found to express both serogroup Y and W135 specificities and were excluded from the analysis to obtain the percent concordance in the results obtained among different laboratories. Therefore, of the 104 attempts to serogroup these 21 *N. meningitidis* isolates, 96 tests gave the correct serogroup, for an overall concordance of 92.3% (96/104 attempts) (Table 3). Concordant results were obtained among all laboratories for the identification of serogroups A (1 isolate), B (5 isolates), C (3 isolates), and Y (2 isolates). Discrepancies were observed for serogroups W135, X, Z, and 29E as well as for the 3 unusual isolates that expressed both serogroup Y and W135 antigenic specificities. The discrepancies involving one laboratory that reported a serogroup W135 meningococcus as a serogroup X organism and the serogroup X isolate as a serogroup W135 organism was most likely the result of a mix-up of the isolates at either the distributing or the receiving laboratory. Three laboratories reported incorrect results for two serogroup Z and two serogroup 29E organisms. One laboratory reported incorrect results for two serogroup Z and two serogroup 29E organisms. This laboratory was using a PCR protocol that allowed the identification of only serogroups A, B, C, W135, X, and Y. Another laboratory reported a serogroup Z organism as nongroupable, because the slide agglutination result was weak for serogroups A, B, W135, Y, and Z, and the PCR protocol used in that laboratory did not allow for the identification of serogroup Z isolates. Finally, one laboratory, using a real-time PCR assay for serogroups A, B, C, W135, X, and Y, reported a serogroup 29E organism as being a serogroup Y organism, which could not be explained. Depending on the methods used, laboratories reported the three unusual strains expressing both Y and W135 antigenic specificities as being either serogroup Y or serogroup W135 strains (Table 4).

DISCUSSION

After 5 years of this QC program, our results indicate a high concordance in the serotyping of *H. influenzae* (98%) and serogrouping of *N. meningitidis* (92%) isolates among the participating laboratories. Some discrepancies were expected due to the subjec-

TABLE 4 Serogrouping results from the five participating laboratories in the International Circumpolar Surveillance program for the three unusual *N. meningitidis* isolates expressing both serogroup Y and W135 antigenic specificities

QC isolate	Serogroup(s) determined (method[s]) by laboratory[a]:				
	1	2	3	4	5
2006-07	W135 (PCR)	W135 (bacterial agglutination)	W135 (bacterial agglutination)	Did not analyze	W135 (bacterial agglutination), Y (PCR)
2008-09	Y (PCR)	W135 (bacterial agglutination)	Y (bacterial agglutination)	W135 (bacterial agglutination)	W135 (bacterial agglutination), Y (PCR)
2008-10	Y (PCR)	NG (bacterial agglutination)	NG (bacterial agglutination), Y (PCR)	W135 (bacterial agglutination and PCR)	W135 (bacterial agglutination), Y (PCR)

[a] NG, nongroupable.

tivity of the slide agglutination test (where the reader determines agglutination visually) and the use of PCR by some laboratories.

The only discrepancies for *H. influenzae* were detected in the first year of the program, and since then, no errors have been found. In one case, a serotype e isolate was misidentified as being nontypeable by the multiplex PCR used (18). Investigation into that case indicated that the multiplex PCR failed to detect the capsule gene, probably due to the interference of the *H. influenzae* capsule-specific primers with primers that target the 16S gene for the identification of *H. influenzae*. Individual PCR setups using the different sets of primers separately showed the presence of the *bexA* gene as well as the serotype e gene in the QC isolate. Subsequently, this laboratory changed their PCR protocol for the identification of *H. influenzae* serotypes from a multiplex PCR to a PCR procedure that uses the different sets of primers separately instead of mixing them up in one reaction. In the other case, the serotype c isolate was misidentified by a slide bacterial agglutination test. Upon the repetition of the test, a positive identification was obtained. Therefore, the actual reason for the original false-negative result was not obvious but might be related to the rare occasions of encountering a less common serotype of *H. influenzae*.

Serogroup determinations for the five most common serogroups of *N. meningitidis* (serogroups A, B, C, Y, and W135) were also accurate for the most part among the participating laboratories, with only one laboratory reporting an error for a serogroup W135 QC organism, most likely due to a mix-up of the specimen. Most of the discordant results involved either rare serogroups (serogroups X, Z, and 29E) or unusual organisms that express two antigenic specificities. The inability to detect the rare serogroups (serogroups X, Z, and 29E) was due mainly to either not using grouping antisera against these serotypes in the slide agglutination test or using PCR protocols that did not include primers to detect them. Many current PCR protocols for the detection and identification of *N. meningitidis* serogroups, including those used by laboratories that failed to identify the correct rare serogroups of *N. meningitidis* in this study, use primers that target either the *ctrA* gene alone or in combination with primers that detect the *sacB* (35) or *mynC* (19) gene for serogroup A, the *siaD* genes for serogroups B and C, and the *synF* and *synG* genes for serogroups Y and W135, respectively (8, 9, 36). As such, serogroups such as serogroups X, Z, and 29E will not be identified by these protocols. However, the QC organisms that had not been correctly identified in the present study were all correctly identified by a PCR protocol that uses primers designed to bind to the unique regions of the *ctrA* genes of strains of serogroups X, Z, and 29E (5).

Our current finding of difficulties in the identification of rare serogroups is in agreement with the results of a previous comparative study of three commercial diagnostic tests for the identification of *N. meningitidis* serogroups, with specificities in the range of 67 to 88% (40). The uncommon serogroups W135, X, Y, Z, and 29E were mainly responsible for the poor performance. Although serogroups X, Z, and 29E are rare causes of invasive meningococcal disease, they have been reported to cause disease (16, 41) or even outbreaks (17). The detection and identification of the relatively rare serogroups X, Z, and 29E based on a PCR assay that targets the sequence variations at the 5′ ends of their *ctrA* genes have been described (5). With common serogroups (serogroups A, C, Y, and W135) being controlled by vaccination, rare serogroups may become relatively more common causes of IMD than before, and therefore, laboratories may wish to adopt and update their laboratory protocols to be able to detect these unusual organisms.

Recently, we (31, 39) as well as others (13) have described a small percentage (1.3%) of serogroup Y and serogroup W135 *N. meningitidis* strains expressing capsules with both serogroup Y and W135 antigenic specificities. Serogroup Y and W135 meningococci have related capsular structures (6), and both are heteropolymers of either glucose linked to sialic acid (for serogroup Y) or galactose linked to sialic acid (for serogroup W135). A number of PCR assays for the differentiation of serogroups Y and W135 have been described (9, 27, 36), but none is based on the detection of nucleotide differences associated with the function of the enzymes responsible for the final capsule structures. As a result, these unusual capsular strains with dual antigenic specificities were classified as serogroup Y strains by PCR (27), but they may show either agglutination in anti-W135 antiserum or agglutination in both anti-Y and anti-W135 antisera (13, 39). The definitive identification of Y-W135 isolates requires the detection of the serine residue at position 310 of the capsule polymerase gene (13).

The serogrouping or serotyping of the capsular antigens of *H. influenzae* and *N. meningitidis* by the slide agglutination test is known to be subjective, technically demanding in skill and experience of the laboratory personnel, and dependent on the quality of the antisera. As a result, discrepancies in the serotyping results for *H. influenzae* and serogrouping results for *N. meningitidis* strains have been reported in the literature. For example, in an Active Bacterial Core (ABC) surveillance study that compared results of slide agglutination tests performed at state health departments to those of capsule typing by PCR performed at the Centers for Disease Control and Prevention, 56 of 141 (40%) invasive *H. influenzae* isolates collected between January 1998 and December

1999 were found to give discrepant results (23). In another study involving 360 invasive *H. influenzae* isolates collected by the Georgia Emerging Infections Program from 1 January 1989 to 31 December 1998, 17.5% of the isolates gave discrepant serotype results when slide agglutination test results were compared to results obtained by PCR capsule typing (34). In a study in Brazil, slide agglutination results obtained either by first screening with an anti-serotype b antiserum followed by testing with antisera against individual serotypes (anti-serotype a, c, d, e, and f antisera) if anti-serotype b antiserum failed to agglutinate the isolate (method 1) or by testing individual isolates with antisera against all six serotypes (anti-serotype a, b, c, d, e, and f antisera) (method 2) were compared to results obtained by capsule typing by PCR (7). In that study, the agreement rate between slide agglutination method 1 or 2 and PCR for invasive isolates was 68% or 88%, respectively. However, for respiratory isolates, the agreement rates between slide agglutination results and PCR typing were 46.5% and 94.2% for methods 1 and 2, respectively (7). For *N. meningitidis*, 58 of 1,298 isolates, or 4.5% of isolates, gave discordant serogrouping results when the results of the slide agglutination test were compared with those of a real-time PCR method (27).

Another source that can contribute to discrepancies lies in the methods used to determine serotyping and serogrouping results. On one hand, the bacterial agglutination test is subjective to read and prone to false-positive results due to the potential cross-reactivities of antisera and is also less sensitive than newer molecular methods such as PCR. However, molecular methods like PCR do not detect the actual capsule but detect only the genes that encode the capsules. Therefore, it is possible that bacteria are truly nontypeable or nongroupable due to either poor expression or the true absence of capsules, but their genes may still be detectable by molecular approaches. This scenario can lead to true discrepancies in the results that are unrelated to poor reagents or techniques for any particular laboratory. An example of this type of discrepancy is the detection of Hib-minus strains, as they carry the gene that encodes the type b capsule but are nonencapsulated due to the loss of their capsule export genes (21). However, in order to detect this strain, both phenotypic and molecular techniques are required, and therefore, quality assurance programs like the one described in this report are essential for surveillance studies to monitor changes in the bacterial population.

Although various methods, such as latex agglutination (25), coagglutination (32), the antiserum agar method (3), countercurrent immunoelectrophoresis (20), and monoclonal antibodies with enzyme-linked immunosorbent assays (ELISAs) (37), have been proposed to improve the determination of the serotype and serogroup antigens, none has gained wide acceptance, possibly because either reliable reagents are not available commercially or the procedure is tedious or requires large volumes of antisera.

Despite the potential problems with the bacterial agglutination test using polyclonal antisera, studies using standardized methods and reagents have proven that the slide agglutination method can still be a very useful and simple tool for the identification of serogroups and serotypes of bacteria (23). Besides the use of standardized reagents and methods, quality control and external proficiency programs are also essential for ensuring the accuracy of testing. Because commercial vendors do not offer external proficiency testing programs for these specialized assays, the ICS Invasive Bacterial Disease Workgroup (IBDWG) incorporated external proficiency testing into the ICS program to ensure the accuracy of data collection for the surveillance of *S. pneumoniae* (30) as well as of *H. influenzae* and *N. meningitidis*.

This QC program has heightened the participating laboratories' awareness of rare *N. meningitidis* serogroups and led to the identification of three unusual *N. meningitidis* strains that might not have been detected (13, 31, 39). As these vaccine-preventable bacterial disease agents continue to evolve either as a result of vaccine pressure or as a response to the natural immunity developed, it is important to continue to monitor the capsular antigens of these invasive bacterial agents. Besides the QC of serotyping of *H. influenzae* and serogrouping of *N. meningitidis*, the IBDWG will also consider in the future the addition of antibiotic susceptibility testing and maybe nucleic acid testing (i.e., nonculture or direct detection of serotypes of *H. influenzae* and serogroups of *N. meningitidis* from clinical specimens) to the current proficiency program.

ACKNOWLEDGMENTS

We thank staff in the participating laboratories for their technical assistance and support of this proficiency program.

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