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Centers for Disease Control Performance Evaluation Program in Bacteriology: 1980 to 1985 Review

CHARLES W. GRIFFIN III,* ELLEN C. COOK, AND MARY A. MEHAFFEY

Microbiology Section, Performance Evaluation Branch, Division of Technology Evaluation and Assistance, Laboratory Program Office, Centers for Disease Control, Atlanta, Georgia 30333

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This report presents a summary and analysis of data from the Centers for Disease Control performance evaluation program in bacteriology for the 6-year period 1980 to 1985. During this period, the number of laboratories enrolled in the program ranged from about 750 to 1,000. Identification results reported by participating laboratories for representative species of six major groups of bacteria were placed into five response categories that were based on the level and accuracy of the identifications. Data on the performance of participants with bacterial groups and performance with selected species within each major group were analyzed. Overall, participants experienced the least difficulty in identifying species or serogroups of members of the gram-positive and gram-negative cocci. Participants encountered greater difficulties with anaerobes, gram-positive bacilli, and miscellaneous gram-negative bacteria. Identification of members of the family *Enterobacteriaceae* was of moderate difficulty. Problems in identifying certain bacterial species were probably related to a number of factors such as the characteristics of the species, its frequency of occurrence, the state of technology available for identification, and the state of proficiency and quality control in individual laboratories at the time of testing. Examples are given of improvements over time in the identification of certain bacterial species. Laboratories participating in an external proficiency testing program should take full advantage of the benefits of participation by instituting measures to correct testing deficiencies identified by the program.

The Centers for Disease Control (CDC) laboratory performance evaluation (PE) program in microbiology represents an expansion of the general laboratory improvement activities that existed some years before the Clinical Laboratories Improvement Act of 1967 delegated certain responsibilities to CDC. As early as the 1930s, the Venereal Disease Research Laboratory at CDC included proficiency testing as part of its efforts to assure good laboratory results. Later, CDC assumed responsibility for the training of state and other health laboratory personnel. As part of this training, and for laboratory improvement, proficiency testing was used to evaluate the quality of laboratory results. When CDC was given new responsibilities under the Clinical Laboratories Improvement Act, various proficiency testing activities were combined into one organization, currently known as the Performance Evaluation Branch in the Division of Technology Evaluation and Assistance, Laboratory Program Office.

Laboratory PEs usually entail the submission of multiple "unknown" samples or specimens to participating laboratories for identification of particular microbial species. The samples are usually prepared and submitted for testing by organizations external to the laboratories, although samples can be prepared and submitted for testing within the quality control system of an individual laboratory (1, 4). PE is in essence an external component of an overall laboratory quality assurance system that should include other components such as (i) training and continuing education of laboratory personnel, (ii) internal quality control, (iii) standardization of laboratory procedures, and (iv) effective management and organization of the laboratory and its procedures.

For about 17 years, the Microbiology Section, Performance Evaluation Branch, CDC, has provided samples for external testing of laboratories in bacteriology. Other testing categories have included mycobacteriology, mycology, parasitology, and virology. Data from the bacteriology program for 1980 to 1985 are summarized and analyzed in this report. During this 6-year period, the number of laboratories enrolled in the bacteriology program ranged from about 750 to 1,000.

MATERIALS AND METHODS

Program design. Shipments of samples to laboratories were usually made quarterly, and each shipment consisted of at least five samples or challenges for identification. As described previously (6), representative samples from each lot or batch of samples, after internal testing at CDC, were sent to a group of selected reference laboratories for validations of their quality before samples were sent to regular participants in the program. Participants were given a specified date for reporting results of their testing to CDC, and each laboratory was sent a PE report after each shipment. During the 6-year period 1980 to 1985, over 100,000 samples containing various microbial species were sent in 24 shipments to an average of 800 laboratories.

Samples for PE. All samples were prepared by CDC personnel and freeze-dried (6, 11). Samples were planned on an annual basis to contain species representative of six major groups of bacteria. The principal bacteria that participants were to identify are listed in Table 1. Other bacterial species (not listed) were included in about half of the samples to represent "normal background flora"; these species were included to challenge selective media or isolation methods or both used by participants. The principal bacterium for identification was the predominant species in mixed-culture type samples.

Each PE sample was identified by a six-character code: the first two characters identified the sample as a microbiol-

* Corresponding author.

TABLE 1. Organisms included in CDC bacteriology PE program (1980 to 1985)

Bacterial group	No. of samples	Principal bacteria[a] included in samples (no. of samples)
Anaerobes	10	*Bacteroides fragilis* (2), *B. melaninogenicus* (1), *B. ovatus* (1), *Clostridium perfringens* (2), *C. difficile* (1), *Fusobacterium mortiferum* (1), *F. nucleatum* (1), *Propionibacterium acnes* (1)
Enterobacteriaceae	39	*Citrobacter diversus* (1), *C. freundii* (1), *Edwardsiella tarda* (1), *Enterobacter agglomerans* (1), *E. cloacae* (1), *E. sakazakii* (1), *Escherichia coli* (3), *Klebsiella pneumoniae* (1), *Providencia rettgeri* (1), *Salmonella anatum* (1), *S. dublin* (2), *S. heidelberg* (2), *S. typhimurium* (6), *Serratia liquefaciens* (1), *S. marcescens* (1), *Shigella boydii* (1), *S. dysenteriae* (3), *S. flexneri* (3), *S. sonnei* (3), *Yersinia enterocolitica* (1), simulated fecal samples devoid of *Salmonella* and *Shigella* spp. (4)
Gram-negative cocci	14	*Neisseria gonorrhoeae* (9), *N. meningitidis* (2), simulated urogenital samples devoid of *N. gonorrhoeae* (3)
Miscellaneous gram-negative bacteria	28	*Acinetobacter calcoaceticus* subsp. *anitratus* (1), *A. calcoaceticus* subsp. *lwoffi* (1), *Achromobacter xylosoxidans* (1), *Aeromonas hydrophila* (1), *Alcaligenes faecalis* (1), *Bordetella bronchiseptica* (1), *B. pertussis* (1), *Campylobacter jejuni* (2), *Flavobacterium meningosepticum* (1), *Gardnerella vaginalis* (1), *Haemophilus influenzae* (2), *H. parainfluenzae* (1), *Moraxella osloensis* (1), *M. nonliquefaciens* (1), *Pasteurella multocida* (2), *Plesiomonas shigelloides* (1), *Pseudomonas aeruginosa* (2), *P. cepacia* (2), *P. fluorescens* (1), *P. maltophilia* (1), *P. putida* (1), *P. stutzeri* (1), *Vibrio parahaemolyticus* (1)
Gram-positive bacilli	5	*Bacillus cereus* (1), *B. megaterium* (1), *Corynebacterium diphtheriae* (1), *C. ulcerans* (1), *Listeria monocytogenes* (1)
Gram-positive cocci	22	*Staphylococcus aureus* (5), *S. saprophyticus* (1), *Streptococcus* group A sp. (2), *Streptococcus* group B sp. (3), *Streptococcus* group C. sp. (1), *Streptococcus* group D sp.-enterococcus (3), *S. bovis* (2), *S. pneumoniae* (3), *S. sanguis* (2)

[a] About half of the samples contained a principal organism mixed with another, or other, microbial species added as background flora.

ogy sample in the subcategory of bacteriology, the third signified the year, the fourth indicated the shipment, and the last two were the number of the sample. For example, sample AB5-C12 was a 1985 sample included in the third quarterly shipment and was number 12 for the year.

Quality control of PE samples. The methods used for the quality control of the bacteriology PE samples have been reported elsewhere (6, 11). After internal testing at CDC, representative vials of all lots or batches of samples were sent first to a group of 8 to 10 reference laboratories for validations of their quality before samples were sent to regular participants. A minimum consensus of 80% by the reference laboratories on the identification of a particular bacterium was required for participants to be held responsible for its identification; in practice, consensus was virtually 100%. The laboratories used for reference purposes were selected on the basis of an excellent history of providing correct identification results.

Laboratory enrollment. During the 6-year period, the number of laboratories enrolled in the bacteriology program ranged from about 750 to 1,000. The enrolled laboratories fell into various descriptive categories and subcategories (6).

PEs. The method of evaluating individual laboratory performance has been described (6). Overall performance of participants with six major groups of bacteria, however, is reviewed in this report. To compare the performance of laboratories with representative species of six major groups of bacteria, responses from the laboratories were placed into five categories based on their reports with individual samples: (i) reported the correct species, group, or type; (ii) reported the correct genus (reported only the genus); (iii) reported the correct genus but an incorrect species, group or type; (iv) reported an incorrect genus or an incorrect genus and species; or (v) failed to isolate the principal organism. For samples containing a particular mixture of microbial species, it was not always possible to determine whether a report of an incorrect genus was a result of misidentification of the principal organism or a failure to isolate the principal organism; however, all reports of an incorrect genus were placed into category iv. Reports from participants of a nonviable sample or reports specifying the absence of the principal organism were placed into category v. In addition, the performance of participants over an 11-year period (1975 to 1985) with 10 different bacterial species was compiled to add the perspective of time to the more current performance analyses.

Information management and analysis. The responses of the participants for representative species were placed into appropriate response categories that were based on the level and accuracy of the identification. The number and percentage of responses were determined for each of five response categories for selected samples included in a particular major group of bacteria. The number of responses and percentages were also determined for each response category with all samples representative of each major bacterial group. Performance with individual samples within each major group of bacteria was then analyzed based on the response categories. Overall performance with the major bacterial groups was also analyzed.

RESULTS

This review provides insight into the types of difficulties experienced by participants in identifying representative members of six major groups of bacteria included in the bacteriology program during the period 1980 to 1985. An overview of performance is presented in Table 2, which shows a spectrum of the relative difficulties laboratories experienced in the isolation and identification of representatives of the six bacterial groups. Overall, the participants

TABLE 2. Spectrum of performance of laboratories in identification of members of six major groups of bacteria over a 6-year period (1980 to 1985)

Laboratory response category	Avg % of responses in response category for:					
	Gram-positive cocci (22)[a]	Gram-negative cocci (14)	*Enterobacteriaceae* (39)	Miscellaneous gram-negative bacteria (28)	Anaerobes (10)	Gram-positive bacilli (5)
Correct species, group, or type	91.0	91.5	83.5	74.1	65.5	48.5
Correct genus (reported only the genus)	1.6	1.6	8.8	7.9	14.4	37.3
Correct genus/incorrect species, group, or type	6.2	1.6	3.3	4.5	4.4	5.3
Incorrect genus or genus and species	1.0	1.1	2.1	9.4	9.6	4.6
Failed to isolate	0.1	4.1	2.3	4.0	6.1	4.4

[a] The number of PE samples for each major group of bacteria is given in parenthesis.

experienced the least difficulty in identifying species or serogroups of members of the gram-positive and gram-negative cocci. Greater difficulties were encountered with anaerobes, gram-positive bacilli, and miscellaneous gram-negative bacteria. Moderate difficulty was experienced in identifying members of the family *Enterobacteriaceae*. It should be emphasized that the percentages of responses shown in Table 2 are averages, so that difficulty in the identification of particular species may not be apparent, especially for a major bacterial group showing a high average percentage of successful identification. Results with individual species representative of the six major groups of bacteria are presented in the following sections.

Gram-positive cocci. For gram-positive cocci, the percentage of responses for the correct species or group ranged from 99.7 for a sample containing *Staphylococcus aureus* to 79.8 for a sample containing *Streptococcus bovis*. Although, overall, participants experienced relatively less difficulty with the gram-positive cocci than with the other five major groups, they encountered the most difficulty in identifying certain organisms within the group: *S. bovis*, *Streptococcus agalactiae*, *Streptococcus sanguis*, and a group C *Streptococcus* species. The results reported for samples containing these streptococci are shown in Table 3.

With the two samples of *S. bovis*, the most common error was misidentification of the bacterium as an enterococcus; 112 participants (13.2%) reported an enterococcus. In addition, about 2% of the participants reported the presence of eight incorrect genera with sample AB1-B07. With *S. agalactiae*, participants experienced more problems with sample AB3-A03 than with two other samples containing the organism. Sample AB3-A03 contained *Corynebacterium xerosis* in addition to the group B streptococcus. Fifty-seven participants (8.5%) reported incorrect streptococcus groups or species, and 20 (3.0%) reported five incorrect genera (of the 20, 13 reported *Staphylococcus epidermidis*). Sample AB5-C14 contained *S. sanguis* (biotype II) in addition to *S. agalactiae*, and participants were requested to identify all bacteria present in the sample. Thirty-one participants (4.7%) reported incorrect streptococcus groups or species (of the 31, 10 reported group G streptococci and 8 reported group C streptococci).

Two samples containing *S. sanguis* (biotype II) were included in the program: sample AB0-A05 contained *S. epidermidis* in addition to *S. sanguis*, and sample AB4-C15 was a pure-culture type sample. The most frequent errors with these samples were reports of incorrect streptococcus groups or species; there were 21 reports in which this viridans group streptococcus was identified as *Streptococcus pneumoniae*, a group D *Streptococcus* species, or as an enterococcus.

In 1980, a bacitracin-susceptible group C *Streptococcus* species was included in a simulated throat culture as an educational review sample (laboratories were not held responsible for their responses). Laboratories were asked to report the presence or absence of group A streptococci, and almost 60% of the participants reported the presence of a group A *Streptococcus* species. Most participants performed

TABLE 3. Performance of laboratories in identification of selected gram-positive cocci

Organism	Sample	Pure (P) or mixed (M)[a]	No. of responses	No. (%) of participants reporting:				
				Correct species or group	Correct genus[b]	Correct genus/ incorrect species or group	Incorrect genus or genus and species	Failed to isolate
Streptococcus bovis	AB0-A04	P	633	512 (80.9)	18 (2.8)	95 (15.0)	8 (1.3)	0 (0.0)
	AB1-B07	P	847	676 (79.8)	25 (3.0)	130 (15.3)	16 (1.9)	0 (0.0)
Streptococcus group B sp.	AB0-A02	P	643	585 (91.0)	43 (6.7)	12 (1.9)	3 (0.5)	0 (0.0)
	AB3-A03	M	671	560 (83.5)	34 (5.1)	57 (8.5)	20 (3.0)	0 (0.0)
	AB5-D21	M	658	604 (91.8)	16 (2.4)	31 (4.7)	1 (0.2)	6 (0.9)
Streptococcus sanguis (biotype II)	AB0-A05	M	636	547 (86.0)[c]	26 (4.1)	55 (8.6)	8 (1.3)	0 (0.0)
	AB4-C15	P	629	537 (85.4)	34 (5.4)	51 (8.1)	7 (1.1)	0 (0.0)
Streptococcus group C sp.	AB0-A06[d]	M	653	248 (38.0)	25 (3.8)	378 (57.9)	2 (0.3)	0 (0.0)

[a] Pure- or mixed-culture type sample.
[b] Reported only the genus identification.
[c] Reports of viridans group included in this response category.
[d] Included as an educational review sample.

TABLE 4. Performance of laboratories in identification of selected gram-negative cocci

Organism	Sample	Pure (P) or mixed (M)[a]	No. of responses	No. (%) of participants reporting: Correct species	Correct genus[b]	Correct genus/ incorrect species	Incorrect genus or genus and species	Failed to isolate
N. gonorrhoeae	AB2-D19[c]	M	665	587 (88.3)	5 (0.8)	0 (0.0)	1 (0.2)	72 (10.8)
	AB5-D18	M	697	670 (96.1)	1 (0.1)	0 (0.0)	0 (0.0)	26 (3.7)
	AB4-C13[d]	M	626	451 (72.0)	36 (5.8)	27 (4.3)	62 (9.9)	50 (8.0)
	AB4-C14	P	651	621 (95.4)	2 (0.3)	5 (0.8)	0 (0.0)	23 (3.5)
	AB5-D19[e]	P	697	620 (89.0)	3 (0.4)	4 (0.6)	1 (0.1)	69 (9.9)
Negative for *N. gonorrhoeae*	AB5-D20[f]	M	694	643 (92.7)	13 (1.9)	38 (5.5)	0 (0.0)	0 (0.0)
N. meningitidis	AB0-C13	P	732	673 (91.9)	28 (3.8)	8 (1.1)	20 (2.7)	3 (0.4)

[a] Pure- or mixed-culture type sample.
[b] Reported only the genus identification.
[c] Sample included *B. catarrhalis*.
[d] Sample included *B. fragilis*.
[e] Sample contained a vancomycin-susceptible strain.
[f] Sample included *N. sicca*.

well with samples containing group A streptococci and with samples containing enterococci; over 98% correctly identified the group A streptococci in two samples, and over 92% correctly identified *Streptococcus faecalis* in three samples.

The percentages of laboratories reporting the correct genus (reported only genus identification) of gram-positive cocci ranged from 0.1% for a sample containing *S. aureus* to 6.7% for a sample containing *S. agalactiae*; both were pure-culture type samples.

Gram-negative cocci. Overall for the gram-negative cocci, participants experienced the most difficulty with mixed-culture type samples containing *Neisseria gonorrhoeae*. Problems in the identification of *N. gonorrhoeae* contained in PE samples were reported previously (5). The results reported by participants for seven selected samples of gram-negative cocci are presented in Table 4. The percentage of laboratories identifying the correct species ranged from 72.0 for a sample (AB4-C13) that contained *N. gonorrhoeae* and *Bacteroides fragilis* to 96.1 for a sample (AB5-D18) that contained *N. gonorrhoeae*, *S. epidermidis*, and *Candida albicans*.

Participants were requested to identify both aerobes and anaerobes in sample AB4-C13, which was described as a peritoneal infection and was suggestive of pelvic inflammatory disease (10). Although about 82% of the participants recognized the presence of a *Neisseria* species in the sample, almost 10% reported incorrect genera such as *Moraxella*; 8% apparently failed to isolate *N. gonorrhoeae* from the sample, possibly because they did not consider its presence. There was no indication of a problem with the quality of the sample: all nine reference laboratories and 17 of 18 referee laboratories were successful in isolating and identifying *N. gonorrhoeae*. One referee laboratory reported an identification of *Moraxella liquefaciens*. The results of thermal degradation testing (11) showed that the concentration of gonococci was $>10^6$ CFU/ml after exposure of freeze-dried vials to 35°C for 7 days. With sample AB5-D18, participants were specifically requested to report the presence or absence of *N. gonorrhoeae*. About 96% of the participants recovered *N. gonorrhoeae* from the sample, but almost 4% did not report its presence. As with the sample discussed above, there was no indication of a quality problem; gonococci were

TABLE 5. Performance of laboratories in identification of selected members of the family *Enterobacteriaceae*

Organism	Sample	Pure (P) or mixed (M)[a]	No. of responses	No. (%) of participants reporting: Correct species, group, or type	Correct genus[b]	Correct genus/ incorrect species, group, or type	Incorrect genus or genus and species	Failed to isolate
Salmonella typhimurium	AB1-B10	M	863	583 (67.6)	208 (24.1)	17 (2.0)	12 (1.4)	43 (5.0)
	AB0-D21	P	821	620 (75.5)	179 (21.8)	17 (2.1)	5 (0.6)	0 (0.0)
Salmonella dublin	AB3-C11	M	649	410 (63.2)	79 (12.2)	146 (22.5)	0 (0.0)	14 (2.2)
	AB3-C12	P	649	423 (65.2)	71 (10.9)	152 (23.4)	3 (0.5)	0 (0.0)
Shigella sonnei	AB3-A01	M	674	560 (83.1)	35 (5.2)	1 (0.1)	8 (1.2)	70 (10.4)
	AB3-A02	P	674	650 (96.4)	16 (2.4)	1 (0.1)	7 (1.0)	0 (0.0)
Shigella dysenteriae	AB0-B08	M	708	538 (76.0)	64 (9.0)	62 (8.8)	11 (1.6)	33 (4.7)
	AB0-B09	P	704	570 (81.0)	53 (7.5)	62 (8.8)	19 (2.7)	0 (0.0)
No *Salmonella* or *Shigella* spp.	AB0-B10[c]	M	683	625 (91.5)	0 (0.0)	0 (0.0)	58 (8.5)	0 (0.0)
Enterobacter agglomerans	AB1-B06	P	875	830 (94.9)	4 (0.5)	4 (0.5)	37 (4.2)	0 (0.0)
Providencia rettgeri	AB2-A04	M	704	662 (94.0)	4 (0.6)	11 (1.6)	27 (3.8)	0 (0.0)
Serratia liquefaciens	AB2-D17	P	657	434 (66.1)	74 (11.3)	75 (11.4)	73 (11.1)	1 (0.2)

[a] Pure- or mixed-culture type sample.
[b] Reported only the genus identification.
[c] Sample contained *Escherichia coli* (A-D group), *Escherichia coli*, *Citrobacter freundii*, and *Staphylococcus epidermidis*.

TABLE 6. Performance of laboratories in identification of selected miscellaneous gram-negative bacteria

Organism	Sample	Pure (P) or mixed (M)[a]	No. of responses	No. (%) of participants reporting:				
				Correct species, group, or type	Correct genus[b]	Correct genus/ incorrect species, group, or type	Incorrect genus or genus and species	Failed to isolate
Acinetobacter calcoaceticus subsp. *anitratus*	AB0-C14	M	749	689 (92.0)	12 (1.6)	5 (0.7)	39 (5.2)	4 (0.5)
Acinetobacter calcoaceticus subsp. *lwoffi*	AB2-B08	M	664	559 (84.2)	17 (2.6)	30 (4.5)	57 (8.6)	1 (0.2)
Alcaligenes faecalis	AB2-B09	P	648	221 (34.1)	122 (18.8)	0 (0.0)	304 (46.9)	1 (0.2)
Achromobacter xylosoxidans	AB2-B10	M	661	427 (64.6)	17 (2.6)	0 (0.0)	217 (32.8)	0 (0.0)
Campylobacter jejuni	AB5-C17	M	603	362 (60.0)	165 (27.4)	16 (2.7)	0 (0.0)	60 (10.0)
Bordetella pertussis	AB5-A06[c]	M	418	220 (52.6)	14 (3.3)	0 (0.0)	1 (0.2)	183 (43.8)
Haemophilus influenzae	AB4-D20	P	650	587 (90.3)	23 (3.5)	24 (3.7)	14 (2.2)	2 (0.3)
Haemophilus parainfluenzae	AB4-A05	P	685	480 (70.1)	107 (15.6)	74 (10.8)	16 (2.3)	8 (1.2)
Moraxella osloensis	AB1-A05	M	765	262 (34.2)	211 (27.6)	21 (2.7)	269 (35.2)	2 (0.3)
Pseudomonas aeruginosa	AB5-D22	P	697	658 (94.4)	15 (2.2)	18 (2.6)	6 (0.9)	0 (0.0)
Pseudomonas cepacia	AB1-D17	P	688	621 (90.3)	21 (3.1)	21 (3.1)	25 (3.6)	0 (0.0)
Pseudomonas fluorescens	AB0-C16	M	751	371 (49.4)	97 (12.9)	250 (33.3)	33 (4.4)	0 (0.0)
Vibrio parahaemolyticus	AB3-B06	M	636	443 (69.7)	39 (6.1)	4 (0.6)	3 (0.5)	147 (23.1)

[a] Pure- or mixed-culture type sample.
[b] Reported only the genus identification.
[c] Included as an educational review sample.

present at a concentration of $>10^6$ CFU/ml after thermal stress. All nine reference laboratories reported *N. gonorrhoeae* for the sample as did 20 of 20 referee laboratories.

Sample AB2-D19 included *N. gonorrhoeae* and *Branhamella catarrhalis*. About 88% of the participants successfully reported the presence of *N. gonorrhoeae*, but almost 11% failed to isolate the organism (failed to report the presence of gonococci). The growth of the particular strain of *B. catarrhalis* included in sample AB2-D19 should have been suppressed on satisfactory lots of selective media, such as Martin-Lewis agar (9).

The results reported by participants for two pure-culture type samples (AB4-C14 and AB5-D19) are also shown in Table 4. Of the participants, 621 (95.4%) identified a typical strain of *N. gonorrhoeae* from sample AB4-C14, but 23 (3.5%) failed to report its presence. Sample AB5-D19 contained a vancomycin-susceptible strain of *N. gonorrhoeae*, and 69 participants (9.9%) failed to isolate and identify the strain.

Participants were requested to report the presence or absence of *N. gonorrhoeae* in a negative sample (AB5-D20); the sample contained *Neisseria sicca*, *S. aureus*, and *Providencia alcalifaciens*. Thirty-two participants (4.6%) reported the presence of *N. gonorrhoeae*, and 6 (0.9%) reported *Neisseria* species other than *N. gonorrhoeae* or *N. sicca*.

A pure culture of *Neisseria meningitidis* was contained in sample AB0-C13, and although about 92% of the participants reported the correct identification, 20 (2.7%) reported incorrect genera: 4 reported *Haemophilus influenzae*, 9 reported *Moraxella* species, and 2 reported *B. catarrhalis*.

The percentage of correct identification reports limited to the genus level ranged from 0.1 for sample AB5-D18 (*N. gonorrhoeae*) to 5.8 for sample AB4-C13 (*N. gonorrhoeae*).

Enterobacteriaceae. With bacteria of the family *Enterobacteriaceae*, the percentage of laboratories that reported the correct species, group, or type ranged from 63.2 for *Salmonella dublin* (sample AB3-C11) to 99.7 for a sample containing *Escherichia coli*. Overall, participants experienced more difficulty in identifying the principal bacterium in mixed-culture type samples than in pure-culture type samples. The performance of laboratories with selected samples containing members of the family *Enterobacteriaceae* is shown in Table 5.

From Table 5, it can be seen that there were some reports of failure to isolate and identify the principal organism from all four mixed-culture type samples containing salmonellae or shigellae; the percentage of laboratories specifically re-

TABLE 7. Performance of laboratories in identification of selected anaerobes

Organism	Sample	Pure (P) or mixed (M)[a]	No. of responses	No. (%) of participants reporting:				
				Correct species or group	Correct genus[b]	Correct genus/ incorrect species or group	Incorrect genus or genus and species	Failed to isolate
Bacteroides fragilis	AB2-C11	M	570	481 (84.4)	47 (8.2)	13 (2.3)	26 (4.6)	3 (0.5)
Bacteroides melaninogenicus	AB4-B07	P	534	374 (70.0)	70 (13.1)	33 (6.2)	49 (9.2)	8 (1.5)
Bacteroides ovatus	AB5-A04	P	563	308 (54.7)	114 (20.2)	123 (21.8)	12 (2.1)	6 (1.1)
Fusobacterium mortiferum	AB2-D16	M	542	242 (44.6)	39 (7.2)	5 (0.9)	253 (46.7)	3 (0.6)
Clostridium perfringens	AB5-C13	P	573	511 (89.2)	44 (7.7)	11 (1.9)	6 (1.0)	1 (0.2)
Clostridium difficile	AB3-D20	P	551	237 (43.0)	196 (35.6)	25 (4.5)	80 (14.5)	13 (2.4)

[a] Pure- or mixed-culture type sample.
[b] Reported only the genus identification.

TABLE 8. Performance of laboratories in identification of selected gram-positive bacilli

Organism	Sample	Pure (P) or mixed (M)[a]	No. of responses	No. (%) of participants reporting:				
				Correct species	Correct genus[b]	Correct genus/ incorrect species	Incorrect genus or genus and species	Failed to isolate
Bacillus cereus	AB4-D18	M	649	206 (31.7)	389 (59.9)	38 (5.9)	16 (2.5)	0 (0.0)
Bacillus megaterium	AB5-A03	P	651	119 (18.3)	449 (69.0)	55 (8.4)	28 (4.3)	0 (0.0)
Corynebacterium diphtheriae	AB4-A01	M	581	360 (62.0)	74 (12.7)	14 (2.4)	0 (0.0)	133 (22.9)
Corynebacterium ulcerans	AB3-C14	M	585	284 (48.5)	227 (38.8)	57 (9.7)	17 (2.9)	0 (0.0)
Listeria monocytogenes	AB2-D20	P	644	539 (83.7)	21 (3.3)	0 (0.0)	81 (12.6)	3 (0.5)

[a] Pure- or mixed-culture type sample.
[b] Reported only the genus identification.

porting the absence of the principal organism (failed to isolate) ranged from 2.2 for *S. dublin* (sample AB3-C11) to 10.4 for *Shigella sonnei* (sample AB3-A01). In comparison, there were no reports of failure to isolate an organism from the four pure-culture type samples containing salmonellae or shigellae. With the latter samples, errors were confined to reports of incorrect genera or incorrect species, groups, or types.

Simulated fecal samples devoid of salmonellae and shigellae were included in the program, and participants were requested to report the presence or absence of the two genera. Sample AB0-B10 is an example of this type of sample and contained *E. coli*, *E. coli* (A-D group), *Citrobacter freundii*, and *S. epidermidis*; about 8% of the participants reported the presence of salmonellae or shigellae, including two who reported *Salmonella typhi* and four who reported *Shigella dysenteriae*.

A pure-culture type sample of *Enterobacter agglomerans* (sample AB1-B06) was successfully identified by about 95% of the participants. However, there were 37 (4.2%) reports of incorrect genera such as *Acinetobacter*, *Aeromonas*, *Hafnia*, *Klebsiella*, *Pasteurella*, and *Shigella*. With a mixed-culture type sample (AB2-A04) containing *Providencia rettgeri* (principal organism) and *S. epidermidis*, 27 participants (3.8%) reported five incorrect genera: *Aeromonas*, *Enterobacter*, *Klebsiella*, *Morganella*, and *Proteus*. Ninety-four percent of the participants reported an identification of *P. rettgeri* for the sample.

Only about 66% of the participants were successful in identifying *Serratia liquefaciens* (sample AB2-D17), which was a pure-culture type sample. Seventy-three participants (11.1%) reported 11 incorrect genera; most of the incorrect reports were *Enterobacter* species (28 reports), *Hafnia alvei* (20 reports), and *Pseudomonas* species (8 reports).

The percentage of laboratories that reported the correct genus (identification limited to genus) ranged from 0.5 for *E. agglomerans* (sample AB1-B06) to 24.1 for *Salmonella typhimurium* (sample AB1-B10). More participants limited their identification to the genus level for the salmonellae than for the other members of the family *Enterobacteriaceae*.

Miscellaneous gram-negative bacteria. Overall, participants experienced more difficulties in identifying miscellaneous gram-negative bacteria than in identifying members of the family *Enterobacteriaceae*. More participants had difficulties with identification at the genus level than with the gram-positive and gram-negative cocci and members of the family *Enterobacteriaceae*. The performance of participants with some selected samples containing miscellaneous gram-negative bacteria is shown in Table 6.

A review of the reports showed that apparently *Acinetobacter calcoaceticus* subsp. *lwoffi* (sample AB2-B08) was more difficult to identify than *A. calcoaceticus* subsp. *anitratus* (sample AB0-C14). Over 4% of the participants reported an identification of *A. calcoaceticus* subsp. *anitratus* for sample AB2-B08, and 8.6% reported 15 incorrect genera. About 4% of the reports of an incorrect genus with sample AB2-B08 were for *Moraxella* species; the next most frequently reported genus was *Pseudomonas*. Participants found a pure-culture type sample of *Alcaligenes faecalis* difficult to identify, and 304 participants (46.9%) reported 13 incorrect genera; most of the identifications reported were members of the genera *Moraxella* and *Pseudomonas*. Participants had difficulty identifying a sample (AB2-B10) with *Achromobacter xylosoxidans*, which also contained *S. epidermidis*. About 33% of the laboratories reported seven incorrect genera, and most of these reports included various *Pseudomonas* species.

Participants were sent a mixed-culture type sample (AB5-C17) containing *Campylobacter jejuni*, which was presented as a simulated fecal sample. The sample was accompanied by a request to report the presence or absence of *Campylobacter* species; 81 participants (11.8%) stated that they did not conduct testing for *Campylobacter* species. Sixty laboratories (10.0%) that did conduct testing failed to isolate the organism.

Sample AB5-A06, which contained *Bordetella pertussis*, was included in the program as an educational review sample. About 39% of the participants indicated that they did not conduct testing for *B. pertussis*. Of the remaining participants that did conduct testing, 183 (43.8%) did not isolate the organism and 14 (3.3%) limited identification to the genus level.

Two pure-culture type samples of *Haemophilus* species were included in the program. Sample AB4-D20 contained *H. influenzae*, which was identified by 90.3% of the participants; 14 participants (2.2%) reported 12 incorrect genera, and 24 (3.7%) reported *Haemophilus* species other than *H. influenzae*. Twenty-three participants (3.5%) limited identification to the genus level. With a sample (AB4-A05) containing *Haemophilus parainfluenzae*, 480 participants (70.1%) reported the correct species identification, 8 failed to isolate the organism, 16 (2.3%) reported nine incorrect genera, and 74 (10.8%) reported a *Haemophilus* species other than *H. parainfluenzae*; 61 (8.9%) reported *H. influenzae*. More participants (about 16%) limited their identification to the genus level with sample AB4-A05 than with sample AB4-D20.

Difficulty was encountered in the identification of *Moraxella osloensis*, which was included in a sample (AB1-A05) with *S. epidermidis*: 269 participants (35.2%) reported 11 incorrect genera, and most of these reports included *Neisseria* species and *B. catarrhalis*. There were 73 (9.5%)

TABLE 9. Participants correctly identifying selected bacterial species in CDC bacteriology PE program (1975 to 1985)

Year	% of participants correctly identifying[a]:									
	Bacteroides fragilis	*Haemophilus influenzae*	*Neisseria gonorrhoeae*	*Neisseria meningitidis*	*Serratia marcescens*	*Streptococcus agalactiae* (group B)	*Streptococcus* sp. (enterococcus, group D)	*Streptococcus pneumoniae*	*Salmonella typhimurium*[b]	*Shigella sonnei*[b]
1975			80 88	83	75				79	
1976			88 87 75 **89**	86		56	68	**93**		80
1977		**76** 72	89 **94**	87	83	50			91	87
1978	69	**86**	**91**			**80**	**78**			
1979			89	82 **88**	**92**		89	95		91
1980	63		91	**88**		**88**	**92**		95 **97**	
1981			93 84						95	
1982	72	**86**	87					97		87
1983	80		93	**96**		84	**93**			87
1984		**90**	**96**		96					
1985			96 **90**			91	**93**		88 **88**	

[a] Percentages in boldface denote results with pure-culture type samples; all other results were with mixed-culture type samples.
[b] Percentages reflect correct identification of genus, genus and group, genus and species, or serotype.

reports of *N. meningitidis* and 53 (6.9%) reports of *B. catarrhalis*. Twenty-one participants (2.7%) reported *Moraxella* species other than *M. osloensis*.

Three samples of *Pseudomonas* species were sent to participants: *P. aeruginosa* (sample AB5-D22), *P. cepacia* (AB1-D17), and *P. fluorescens* (sample AB0-C16). The first two were pure-culture type samples, and the third contained a mixture of *P. fluorescens and S. epidermidis*. Most participants performed well in the identification of *P. aeruginosa* and *P. cepacia*. However, 33 participants (4.4%) reported nine incorrect genera for the sample containing *P. fluorescens*, and 250 (33.3%) reported *Pseudomonas* species other than *P. fluorescens*; 106 (14.1%) reported *P. aeruginosa*.

Participants were asked to determine the presence or absence of *Shigella* or *Vibrio* species or both in sample AB3-B06, which contained *Vibrio parahaemolyticus*, *E. coli*, *H. alvei*, *Morganella morganii*, and *Streptococcus faecium*. Although over 75% of the participants recognized the presence of a *Vibrio* species in the sample, 23.1% (147) failed to do so. A higher percentage of participants limited their identification to the genus level with samples containing *C. jejuni*, *M. osloensis*, *A. faecalis*, and *H. parainfluenzae*. The percentage of laboratories that reported the correct genus (identification limited to genus) ranged from 1.6 for *A. calcoaceticus* subsp. *anitratus* to 27.6 for *M. osloensis*.

Anaerobes. In the instructions accompanying all 10 of the samples containing anaerobes, participants were either alerted to the possibility of their presence or specifically requested to identify them. Laboratories had the greatest success in identifying *Clostridium perfringens* and *B. fragilis* (Table 7), although 26 participants (4.6%) reported eight incorrect genera for the latter sample. Participants had more difficulty in identifying *Bacteroides melaninogenicus* and *Bacteroides ovatus* than in identifying *B. fragilis*. Forty-nine participants (9.2%) reported 17 incorrect genera, of which *Capnocytophaga* was the most frequent, for the sample (AB4-B07) containing *B. melaninogenicus*. More participants made errors at the species level in the identification of *B. ovatus*.

Although 83% of the participants recognized the presence of a *Clostridium* species in sample AB3-D20, which contained *Clostridium difficile*, only 43% correctly identified the species and 80 participants (14.5%) reported the presence of eight incorrect genera for this pure-culture type sample. The most frequently reported incorrect genus was *Eubacterium* (40 reports), followed by *Fusobacterium* (13 reports) and *Bacteroides* (11 reports).

Participants experienced the greatest difficulty with a sample (AB2-D16) that contained *Fusobacterium mortiferum* and *Bacillus cereus*: 253 participants (46.7%) reported five incorrect genera. The most frequently reported incorrect genus was *Bacteroides* (106 participants [19.6%] reported *B. ovatus*).

With this group of bacteria, the percentage of participants that reported the correct genus (identification limited to genus) ranged from 7.2 for *F. mortiferum* to 35.6 for *C. difficile*.

Gram-positive bacilli. Overall, more participants limited their identification to the genus level with gram-positive bacilli than with the other five major groups. Performance with five samples containing representatives of the gram-positive bacillus group is shown in Table 8. As might be anticipated, participants had considerable difficulty isolating and identifying *Corynebacterium diphtheriae*: 133 participants (22.9%) failed to report the presence of the organism in sample AB4-A01. Participants were requested to report the presence or absence of *C. diphtheriae* in a sample that contained *N. sicca*, *S. aureus*, *S. epidermidis*, and *S. sanguis* in addition to *C. diphtheriae*. Although 458 participants (78.8%) recognized the presence of a *Corynebacterium* species, only 360 (62.0%) reported the correct species. Participants experienced difficulty in identifying *Corynebacterium ulcerans* in a sample (AB3-C14) that also contained *S. epidermidis* and *S. sanguis*. Of the 568 participants (97.0%) that recognized a *Corynebacterium* species in the sample, 248 (48.5%) reported *C. ulcerans* and 227 (38.8%) reported the correct genus (identification limited to genus). Fifty-seven participants (9.7%) reported species other than *C. ulcerans*, including 37 (6.3%) who reported *C. diphtheriae*; 17 participants (2.9%) reported four incorrect genera.

Most of the responses for the two samples containing *Bacillus* species were limited to genus identification, as might have been foreseen. However, 220 (33.8%) and 260 (39.9%) participants reported "*Bacillus* species, not *B. anthracis*" for sample AB4-D18 (*B. cereus*) and sample AB5-A03 (*Bacillus megaterium*), respectively. Twenty-eight participants (4.3%) reported the presence of 10 incorrect genera for sample AB5-A03, and 16 (2.5%) reported eight incorrect genera for sample AB4-D18. Twenty-two participants (3.4%) misidentified *B. megaterium* (sample AB5-A03) as *Bacillus anthracis*.

Participants had more success with the identification of *Listeria monocytogenes* (sample AB2-D20) than with the identification of the other gram-positive bacilli: 539 participants (83.7%) reported the correct species. However, 81 participants (12.6%) reported five incorrect genera, with 42 reports of an enterococcus.

Historical perspective of performance. There are limitations on the number of PE samples that participants can reasonably be expected to test annually. The submission of an inordinate number of samples to participants for testing could add significantly to their work load and costs, especially for small laboratories. Because of limitations on the number of samples for testing, some bacterial species were included in the program too infrequently for an assessment of patterns of performance. Examples of patterns of improvement in identification are presented in Table 9, in which the performance of participants during the period 1975 to 1985 with 10 different bacterial species is shown. Improvement in the identification of most of the species is apparent. However, there appeared to be some regression in performance with *S. typhimurium* in 1985; we are unable to offer an explanation for these results.

DISCUSSION

It should be recognized that individual participants and individual laboratories enrolled in the CDC bacteriology program changed during the six-year period reviewed in this report. Although some of the laboratories changed from year to year, over 60% of the laboratories enrolled in the program in 1980 were still on the enrollment list in 1985. No data are available relating to the turnover of laboratory personnel that conducted the testing of the PE samples during the period 1980 to 1985. New technology in the form of certain identification kits and systems were introduced into the clinical laboratory testing area during the period covered by this report. All of these factors, and others, had an impact on the results obtained by participants with the PE samples for any one testing event during the 6-year period. The examples of improved performance (1975 to 85) in the identification of certain species possibly reflect several contributing factors: (i) a change in participants from year to year, in which some poor performers may have withdrawn from the program and been replaced by better-performing laboratories; (ii) improvement in the performance of individual participants; and, (iii) introduction of new or improved technology. Also, for those participants who remained in the program for years, the benefit of repeated testing with identical or similar PE samples probably played some role in instances in which improvements in performance were noted.

The difficulties observed for laboratories in testing certain PE samples probably supports what has been observed on a smaller scale (in individual laboratories) or what has been generally perceived to be the relative level of performance with certain bacterial species in actual specimens from patients. For example, there is probably a general perception that a large majority of laboratories perform well in the identification of group A streptococci from pharyngeal specimens, and participants performed very well in their identification in PE samples. Also, there is probably a general perception that laboratories do not perform as well with certain miscellaneous gram-negative bacteria or with anaerobes. The PE data presented here seem to support these general perceptions.

Difficulties in the identification of certain species were probably related to a number of factors, some of which were noted previously (7), such as (i) the characteristics of particular species (and strains), such as relatedness to other organisms, (ii) the frequency of their occurrence, (iii) the frequency with which they are encountered in different populations of patients, (iv) the state of the technology available for their identification, (v) the state of proficiency in individual laboratories at any given time, (vi) quality control and quality assurance systems in use in individual laboratories, and (vii) supervisory and management practices.

It seems apparent from the PE data that some species not commonly encountered in specimens from patients are often misidentified as other species or as belonging to other genera; three examples are *M. osloensis*, *P. fluorescens*, and *F. mortiferum*. Misidentification of these organisms, and others with a low frequency of occurrence, may contribute somewhat to an observed frequency of occurrence lower than their actual frequency of occurrence. Some participants in the CDC PE program may not have encountered infrequently occurring species in the populations of patients they serve or may not have recognized the species when they encountered it. Perhaps to circumvent the possibility of misidentification or to circumvent the expertise needed to identify certain species, some laboratories may limit their identification to the genus level; some laboratories may limit their identification to the genus level but eliminate the possibility of certain species. A report of "*Pseudomonas* sp., not *P. aeruginosa*" would be an example of the latter case. Laboratories that limit their identification to some species of the genus level may refer such isolates to another laboratory for further identification. The clinical relevance to

the treatment of a patient of information gained by identifying certain bacteria to the species level is controversial (7, 8).

The failure to detect or isolate a principal bacterium from a PE sample should be of particular concern to a laboratory. This type of error indicates a problem in the initial steps of the process of isolation and identification, such as (i) improper handling of the sample, e.g., delay in testing; (ii) use of an inappropriate medium for inoculation; (iii) use of a medium with poor selectivity, e.g., a medium that has deteriorated or was improperly prepared; (iv) failure to obtain discrete colonies, e.g., an improperly streaked plate; (v) failure to select correct colonies for further identification; and (vi) use of a mixed culture for further identification, e.g., failure to use care in selecting growth for the primary medium (2). Failure to isolate the principal organism from PE samples was more pronounced with samples containing *B. pertussis*, *V. parahaemolyticus*, *C. diphtheriae*, *C. jejuni*, and *N. gonorrhoeae*, and with *Salmonella* and *Shigella* species.

Proficiency testing can be a useful means of detecting and confirming inadequacies in laboratory testing (3, 4, 12), even though the measurement of performance of laboratories with external PE samples is relative and may not represent performance in actual practice. External proficiency testing can be used to complement the internal quality control or quality assurance system of an individual laboratory. An internal quality control program is generally more appropriate for detecting day-to-day testing errors, but an external program can sometimes detect errors that may not otherwise be identified. The types of errors detected by an external program are usually chronic, e.g., the use of inadequate methods and culture media or consistent problems with the identification of particular species. Unfortunately, some laboratories do not take full advantage of the benefits of an external program for improving performance. For example, after the identity of the contents of PE samples are made available to participants, and if a problem is apparent, individual participants should take corrective action. If a laboratory fails to isolate an organism, it may wish to request an additional sample for retesting. If a laboratory misidentified an isolate, tests can be repeated and quality control procedures can be reviewed. Periodic critiques can be scheduled to discuss problems and to identify whether there is a need for revision of procedures or for training. Individual laboratories can retain copies of their PEs for periodic reviews to determine whether chronic problems exist in the isolation and identification of certain species or groups of organisms. There is sufficient volume of a culture provided in each PE sample to divide among a number of laboratory personnel for independent testing, if this approach to continuing education is practical for the laboratory. This suggestion is probably more applicable to larger laboratories and to laboratories that have personnel who conduct testing after the usual hours of operation of the laboratory. Similarly, laboratories can conduct their own internal proficiency testing by using cultures derived from an external program or from other sources. The advantage of using cultures from an external program is that internal testing results can be compared with those obtained by many other laboratories with the same strain.

Laboratories that cannot achieve and maintain proficiency in identifying particular species should consider alternatives for improving the quality of their bacteriology services. One alternative is to limit services to those procedures that are performed well, so that accurate and clinically useful information is made available to the users of laboratory services.

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