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Five Years of Experience with a National External Quality Control Program for the Culture and Identification of *Neisseria gonorrhoeae*

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In response to a need for monitoring the proficiency of public health laboratories in isolating and identifying *Neisseria gonorrhoeae*, a national external quality control program was developed. Essentially, three types of freeze-dried samples, representing different levels of challenge for identification, were sent to laboratories for testing. The quality of the samples was confirmed by external reference laboratories, and stability of the samples was confirmed by thermal degradation tests before the samples were sent to laboratories enrolled in the program. By analyzing laboratory results, we identified common errors and chronic problems in testing samples. As a group, laboratories testing small numbers of actual patient specimens did not perform as well in the program as did laboratories testing large numbers of specimens; however, the performance of laboratories testing small numbers of specimens improved over time. Overall, laboratories experienced the most difficulty with samples containing *N. gonorrhoeae* mixed with other microbial species. Laboratories that performed confirmatory tests committed fewer errors than did laboratories that performed presumptive tests only, but the failure to use pure cultures of gonococci for inoculation of cystine tryptic digest agar appeared to be a chronic problem in confirmatory carbohydrate testing. A review of the use of different plating media and confirmatory tests showed that the use of certain media and tests changed over time.

In 1976, the Centers for Disease Control (CDC) initiated an external quality control program for the culture and identification of *Neisseria gonorrhoeae*. It was developed by the Microbiology Section, Proficiency Testing Branch (now the Performance Evaluation Branch, organizationally a part of the Laboratory Program Office), in response to a request from the Venereal Disease Control Division (now part of the Center for Prevention Services). This Division recognized a need for the program because (i) the monitoring of laboratory proficiency in isolating and identifying *N. gonorrhoeae* is a critical aspect of operational quality control in gonorrhea screening programs, and (ii) the monitoring systems of state and city venereal disease control programs were in various stages of development. We asked the State Public Health Laboratory Directors to identify the state laboratories that would enroll in the CDC program and to inform us of the approximate number of specimens each laboratory tested annually for *N. gonorrhoeae*.

Various types of testing samples were designed to afford different levels of challenge to the laboratories enrolled in the program. In addition to typical strains of gonococci, penicillinase-producing (PP) *N. gonorrhoeae*, carbon dioxide-dependent strains, and a vancomycin-susceptible (VS) *N. gonorrhoeae* strain were included in the samples.

A data management system was designed to provide summaries of test results, which enabled laboratories to compare performance data and methods. This report summarizes testing results from the first 5 years of operation of the CDC program.

MATERIALS AND METHODS

Sample planning and types of samples. Essentially three types of freeze-dried samples, representing different levels of challenge for isolation and identification, were included in the program: (i) samples containing only *N. gonorrhoeae*, designated pure-type samples; (ii) samples containing *N. gonorrhoeae* as the predominant species mixed with other microbial species, designated mixture-type samples; and (iii) samples containing microbial species other than *N. gonorrhoeae* or *Moraxella* species, designated negative-type samples. Samples containing *N. gonorrhoeae* consisted of carbon dioxide-dependent strains, PP *N. gonor-*

rhoeae, a VS strain, and typical strains. Microbial species other than *N. gonorrhoeae* included in samples were *Acinetobacter calcoaceticus* var. *lwoffi*, *Branhamella catarrhalis*, *Candida albicans*, *Corynebacterium* sp., members of the *Enterobacteriaceae*, *Gardnerella vaginalis*, *Listeria monocytogenes*, *Neisseria lactamica*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* (group A and B), *Streptococcus faecalis*, *Streptococcus sanguis*, and anaerobes (*Bacteroides fragilis*, *Clostridium sporogenes*, and *Peptostreptococcus anaerobius*). With a few exceptions, such as with *Proteus* species, the growth of organisms added to mixture-type samples as "background flora" was suppressed on satisfactory lots of modified Thayer-Martin agar.

Each proficiency testing sample was accompanied by an anatomic site source description. Of the samples containing *N. gonorrhoeae*, with the exception of one throat specimen and two anal specimens, all were described as either cervical or urethral specimens.

Internal acceptance specifications were developed to assure the quality of the freeze-dried samples. (i) Thermal degradation tests were performed to assure that samples containing *N. gonorrhoeae* had a minimum of 10^5 to 10^6 CFU of gonococci per ml after exposure to 35°C for 2 weeks (except for two samples specially prepared to contain about 10^4 to 10^5 CFU/ml so as to present a greater challenge for detection). (ii) Mixture-type samples contained appropriate species consistent with the clinical source description, with *N. gonorrhoeae* as the predominant species. (iii) The results of characterization tests essential for identification were satisfactory. (iv) The residual moisture content of the samples did not exceed 1% by the method employed. A group of four external reference laboratories was used to validate the CDC results with each sample. The reference laboratories were public health laboratories that conducted large volumes of tests for *N. gonorrhoeae*.

Laboratory enrollment. The Venereal Disease Control Division, Center for Prevention Services, CDC, initially identified the gonorrhea-screening laboratories for each state to be enrolled in the program. A small number of other laboratories enrolled because of an interest in the quality control aspects of the program. The number of laboratories in the program increased from about 250 in 1976 to about 430 in 1980. Information was obtained from over 250 laboratories concerning their routine workload in testing actual patient specimens for *N. gonorrhoeae*.

RESULTS

Report period. The proficiency testing results reported here are from the period 1976 through 1980, during which about 15,000 freeze-dried samples were sent to laboratories throughout the United States in nine scheduled shipments.

Results from reference laboratories. All reference laboratories successfully isolated and identified *N. gonorrhoeae* from the 35 samples prepared at CDC that contained different strains of the bacterium. All reference laboratories agreed that gonococci were not present in the 10 negative samples, with the exception of one sample that contained *C. albicans*, *S. epidermidis*, and *B. fragilis*. One reference laboratory reported, primarily on the basis of fluorescent antibody (FA) staining, that the sample contained *N. gonorrhoeae*; two other reference laboratories that performed FA staining reported a negative result. Three (1.1%) of 272 participating laboratories reported that the sample contained *N. gonorrhoeae*—one on the basis of presumptive testing and two on the basis of confirmatory testing (neither employed FA staining). Nineteen participating laboratories performed FA staining with the sample, and all reported a negative result. Internal testing at CDC with additional vials of the sample failed to detect the presence of gonococci.

Presumptive testing. Overall, the percentage of participant laboratories using presumptive testing only and reporting correct results with the 35 samples containing *N. gonorrhoeae* ranged from 62.6 to 98.7%. The average percentage of laboratories giving correct presumptive reports for the 18 samples which contained *N. gonorrhoeae* mixed with other species was 85%. The average percentage of laboratories giving correct presumptive reports for the 17 samples which contained only gonococci was 93%. The difference between these average percentages was statistically significant ($P < 0.01$), indicating, as might be anticipated, that laboratories experienced more difficulty with the mixture-type samples than with the pure-type samples.

Primary plating media. Beginning with the 1977 II shipment, the report form used by participants was modified so that results could be reported for a greater variety of plating media. Participants reported use of the following media: Thayer-Martin agar (20), modified Thayer-Martin agar (14), improved Thayer-Martin (Martin-Lewis) agar (15), New York City medium (8), Transgrow medium (15), and chocolate agar. Because of the very small number of reports on the use of New York City medium, no results from use of this medium have been presented.

Growth or no growth on plating media could be reported by participants on the report form, and the presumptive identity of growth as *N. gonorrhoeae* depended upon a laboratory giving a positive presumptive report. Therefore, to evaluate the results reported by participants for various media, the percentage of positive presumptive reports from laboratories conducting presumptive testing only with pure-type and mixture-type samples was compiled according to single or multiple use of plating media.

As noted previously, the percentage of positive presumptive tests with samples containing only *N. gonorrhoeae* was higher than that with samples containing a mixture of species. The highest percentage of positive presumptive tests

TABLE 1. Comparison of CU and other confirmatory test results reported by participating laboratories with samples containing *N. gonorrhoeae* mixed with other species and with samples containing only *N. gonorrhoeae*

Type sample	No. positive/no. tested (% positive)						
	CU test[a]				ONPG	FA	CA
	Glucose	Maltose	Sucrose	Lactose			
17 samples containing *N. gonorrhoeae* only	3,174/3,321 (95.6)	54/3,296 (1.6)	30/3,131 (0.9)	15/2,186 (0.7)	5/1,148 (0.4)	1,143/1,194 (95.7)	157/160 (98.1)
18 samples containing *N. gonorrhoeae* and other species	3,329/3,415 (97.5)	150/3,387 (4.4)	91/3,146 (2.9)	63/2,231 (2.8)	13/1,109 (1.2)	1,213/1,270 (95.5)	113/115 (98.2)

[a] CTA, Minitek (BBL Microbiology Systems), phenol red broth, and rapid fermentation test.

was obtained with improved Thayer-Martin (Martin-Lewis) agar, and the lowest number was obtained with chocolate agar when samples of *N. gonorrhoeae* mixed with other microbial species were tested; single use of improved Thayer-Martin agar increased from about 5% in 1977–1978 to about 28% in 1979–1980. Some laboratories reported the use of three or more different media for testing each sample, but presumably a laboratory would not use this number of media routinely for actual clinical specimens. The routine use of more than two media for the primary plating of gonorrhea specimens is not recommended, nor does the procedure appear to be cost effective. During 1979–1980, about 92% of the reports were of the use of one or two media; 49% for one medium and 43% for two media. In the latter case, 92% of the reports were of the use of chocolate agar in combination with a selective medium; modified Thayer-Martin and chocolate agar represented 45% of the use of two media. The use of improved Thayer-Martin in combination with chocolate agar increased from 5% (1977–1978) to 22% (1979–1980). During 1979–1980, the use of a single medium and the use of three media decreased by about 6 and 22%, respectively, whereas the use of two media increased by about 16%. The use of chocolate agar in combination with one or more selective medium increased by about 12%.

Confirmatory testing. The percentage of laboratories that did confirmatory testing with the 35 samples containing *N. gonorrhoeae* and that reported the presence of *N. gonorrhoeae* ranged from 85.2 to 99.2%.

Overall, the average percentage of laboratories giving correct confirmatory reports was 94.0% with mixture-type samples and 96.0% with the pure-type samples. The difference between these average percentages was not statistically significant ($P > 0.70$). This finding, coupled with the fact that a significant difference was found between the average percentage of errors with mixture-type samples and pure-type samples in presumptive testing, indicates that confirmatory testing did reduce the errors laboratories experienced with mixture-type samples.

A comparison of confirmatory test results for samples containing *N. gonorrhoeae* mixed with other species and for samples containing only *N. gonorrhoeae* is shown in Table 1. The number and percentage of erroneous positive results with the carbohydrate tests (maltose, sucrose, and lactose) and with *ortho*-nitrophenyl-beta-D-galactopyranoside (ONPG) for the 18 mixture-type samples were higher than those for the 17 pure-type samples. No differences were apparent in the results reported for the glucose, FA, and coagglutination (CA; Phadebact Gonococcus Test; Pharmacia Diagnostics, Piscataway,

N.J.) tests with the above two types of samples.

The 18 mixture-type samples contained one or more species capable of utilizing some or all of the three carbohydrate substrates (maltose, sucrose, and lactose) used in confirmatory testing. A comparison of the percentage of erroneous positive test results reported for the three carbohydrates according to the number of fermenters present in various samples is shown in Table 2. The percentage of erroneous results with the three carbohydrates was lowest with the 17 samples containing only gonococci as indicated in Table 1.

Beginning with the 1977 II survey, confirmatory carbohydrate test results were compiled according to the method/medium reported by each participating laboratory. From the 1977 survey through the 1980 II survey, 12 samples containing *N. gonorrhoeae* only and 15 samples containing a mixture of *N. gonorrhoeae* and other species were mailed to laboratories; the confirmatory carbohydrate test results reported for these samples according to the method/medium used are shown in Table 3.

The percentage of erroneous positive reactions reported for maltose, sucrose, and lactose/ONPG was observed to be higher with the 15 mixture-type samples than with the 12 samples containing only gonococci, with the exception of phenol red broth for maltose and the BACTEC (BBL Microbiology Systems, Cockeysville, Md.) method with maltose. The latter method utilized fructose (not shown in tables) and did not include sucrose and lactose. These differences were statistically significant ($P < 0.05$) only for the reports with cystine tryptic digest agar (CTA) for all three carbohydrates and for reports with the rapid fermentation test for maltose. The smaller number of tests reported for other methods may explain why significant differences were not found with some of these methods.

Regarding glucose, the number of correct positive reports ranged from 90.9% for phenol red broth to 100.0% for the BACTEC method for the 12 samples which contained only gonococci. Since the number of tests reported for these two methods was very limited, the range of positive results with samples containing only gonococci was 94.4% for CTA to 98.3% for the Minitek system if the results of the phenol red broth and BACTEC methods are not considered. With samples containing a mixture of species, the range was 97.3% for CTA to 99.1% for the rapid fermentation test.

Although the number of tests reported in which the CA method was used was less than the number of FA tests reported (Table 1), the percentages of positive results for both methods were comparable. With the nine samples devoid of gonococci, 6 (4.7%) of 129 FA tests were reported as positive; 3 of the 6 positive tests were reported for a sample which contained only *S. aureus*. Four (5.1%) of 78 FA test results were reported as positive for a sample which contained *N. meningitidis*. There were 11 CA tests reported for four samples devoid of gonococci, with one positive test result.

The use of different confirmatory testing methods by laboratories participating in the program changed over time. In the carbohydrate utilization (CU) test group, the use of CTA with carbohydrate substrates declined by about 15% between 1977–1978 and 1979–1980. The use of RFT and the Minitek method increased by 20 and 38%, respectively; use of the BACTEC method increased from 1 to 2%.

The use of the FA method declined slightly (about 6%) between 1977–1978 and 1979–1980. With reference to CA tests, the Phadebact Gonococcus Test was not commercially available during 1977–1978, but it represented about 7% of the 1979–1980 reports. Initial reports on the use of CA tests were received in 1979. Overall, among the three confirmatory test groups (CU, FA, and CA), the use of CU tests and the FA method declined somewhat, 4 and 6%, respectively.

The FA method constituted about 32% of the confirmatory tests performed during 1977–1978,

TABLE 2. Comparison of erroneous positive carbohydrate test results with the number of maltose, sucrose, or lactose fermenters present in samples containing *N. gonorrhoeae* mixed with other microbial species

No. of fermenters[a]	Carbohydrate test								
	Maltose			Sucrose			Lactose		
	No. of samples	No. positive/ no. of tests	% Positive	No. of samples	No. positive/ no. of tests	% Positive	No. of samples	No. positive/ no. of tests	% Positive
0	17	54/3,296	1.6	17	30/3,131	0.9	17	15/2,186	0.7
1	4	31/760	4.1	3	8/520	1.5	10	25/1,223	2.0
2	12	109/2,266	4.8	14	74/2,434	3.0	5	21/625	3.4
3	2	10/361	2.8	1	9/192	4.7	3	17/383	4.4

[a] Number of maltose, sucrose, or lactose fermenters in the samples.

TABLE 3. Confirmatory carbohydrate utilization test results according to the method/medium reported for 12 samples containing *N. gonorrhoeae* and for 16 samples containing a mixture of *N. gonorrhoeae* and other species

Method/medium	Sample type	No. of positive/no. of tests (% positive)			
		Glucose	Maltose	Sucrose	Lactose/ONPG
CTA	Pure	1,190/1,260 (94.4)	25/1,262 (2.0)	8/1,198 (0.7)	8/1,067 (0.7)
	Mixture	1,532/1,574 (97.3)	58/1,545 (3.8)	38/1,453 (2.6)	27/1,310 (2.1)
Phenol red broth	Pure	10/11 (90.9)	3/11 (27.3)	0/11 (0.0)	0/10 (0.0)
	Mixture	25/27 (92.6)	3/27 (11.1)	3/28 (10.7)	2/26 (7.7)
Rapid fermentation test	Pure	519/529 (98.1)	0/524 (0.0)	1/403 (0.3)	1/247 (0.4)
	Mixture	590/596 (99.1)	15/593 (2.5)	5/525 (1.0)	6/295 (2.0)
Minitek[a]	Pure	349/355 (98.3)	6/351 (1.7)	6/356 (1.7)	1/278 (0.4)
	Mixture	391/397 (98.5)	18/381 (4.7)	15/385 (3.9)	5/309 (1.6)
BACTEC[b]	Pure	24/24 (100.0)	0/24 (0.0)		
	Mixture	27/27 (100.0)	0/26 (0.0)		

[a] ONPG is used in the Minitek System (BBL).
[b] BACTEC (BBL) radiometric method.

with 9% of its use being in combination with CU tests and 23% as the only confirmatory test performed. During 1979–1980, FA and CA tests constituted about 37% of the confirmatory tests, about 11% of their use being in combination with CU tests and 26% as the only confirmatory test performed.

Extent of presumptive and confirmatory testing errors. A comparison of the number and percentage of errors according to presumptive and confirmatory reports for pure-type and mixture-type samples containing *N. gonorrhoeae* is shown in Table 4. Two listings of pure-type samples, with and without the sample that contained the VS *N. gonorrhoeae* strain, are shown because of the high error with this sample.

In the table, an error was defined as a negative report (the failure to report the presence of *N. gonorrhoeae* either by presumptive or confirmatory testing). Analyses of differences in the percent error for presumptive testing and for confirmatory testing with pure-type and mixture-type samples showed these differences to be statistically significant.

There were only 39 reports of no growth on primary plating media with the 16 pure-type samples (not including the VS *N. gonorrhoeae* sample), which represented only about 0.8% of the 4,922 individual vials that constituted the 16 samples mailed to the laboratories. It is difficult to determine with absolute certainty whether the gonococci were actually nonviable in every report of no growth or whether other factors, such as media quality or incubation conditions, were responsible for such reports. In internal quality control testing at CDC with the 16 pure-type samples, gonococci were recovered in sufficient numbers for identification from all vials subjected to thermal-degradation tests, and all reference laboratories successfully recovered the bacterium.

PP *N. gonorrhoeae*. During the course of the program, four samples containing PP *N. gonorrhoeae* were mailed to laboratories with instructions to test for beta-lactamase by the method used routinely. A summary of the results reported by the laboratories for the four samples is presented in Table 5. For the samples mailed in 1977, about 60% of the laboratories reported use of the agar disk diffusion method (21) and about 40% reported the use of chemical methods (1). The percentage of laboratories using either the agar disk diffusion or chemical method was about equal in 1980. Of the three chemical

TABLE 4. Comparison of errors according to presumptive and confirmatory reports for samples containing only *N. gonorrhoeae* and with samples containing *N. gonorrhoeae* mixed with other microbial species

Sample type	No.	No. negative[a]/no. of reports (% negative)		Probability (*t* test)
		Presumptive testing	Confirmatory testing	
Pure	17[b]	92/1,255 (7.3)	177/3,999 (4.4)	$P < 0.0002$
Pure	16[c]	72/1,174 (6.1)	139/3,748 (3.7)	$P < 0.0008$
Mixture	15[d]	166/1,176 (14.1)	201/3,317 (6.1)	$P < 0.0001$

[a] Did not report presence of *N. gonorrhoeae*.
[b] Including VS *N. gonorrhoeae* strain.
[c] Not including VS *N. gonorrhoeae* strain.
[d] Not including three samples with source description of throat or anal.

methods used, overall the chromogenic cephalosporin method appeared to be the most sensitive, but only about 27% of the laboratories performing chemical tests reported use of this method in 1980. In 1977, the laboratories also tested two samples containing non-PP *N. gonorrhoeae* strains (not shown in Table 5). About 4.6% of the tests were reported as positive for beta-lactamase by laboratories using the agar disk diffusion method. About 4.4% were reported as positive by laboratories using chemical methods, but only by laboratories using the iodometric method.

VS *N. gonorrhoeae*. A VS *N. gonorrhoeae* strain was sent to laboratories in 1980. Instructions accompanying the sample recommended that chocolate agar be inoculated in parallel with a selective medium. Laboratories performing presumptive testing only, as well as laboratories using confirmatory testing, experienced difficulties with the strain. About 25% of the laboratories performing presumptive testing only and about 15% of those performing confirmatory testing were not successful in recognizing the VS *N. gonorrhoeae* strain. The number (4.8%) of reports of no growth on samples without chocolate agar exceeded the number (1.2%) of reports of no growth on samples in which chocolate agar was used alone or in combination with selective media for plating of the VS *N. gonorrhoeae* strain.

Workload and tracking of performance. As mentioned previously, information relating to the number of actual patient specimens examined for *N. gonorrhoeae* was obtained from over 250 laboratories. Initially in the program, the group of laboratories (subgroup A) testing the smallest number of patient specimens (0 to 12 per day) was identified as having the lowest performance of the laboratory subgroups studied. For this reason, the performance of subgroup A laboratories was tracked over time in the program and compared with the performance of the group of laboratories testing over 100 specimens per day (subgroup D) and with State Health Department laboratories (subgroup E). The workloads of subgroup E laboratories were not considered because the performance of this subgroup was chosen as a reference point for comparison with that of other subgroups. Overall, the mixture-type sample represented the more difficult challenge for identification of *N. gonorrhoeae*, and the performance of the above three subgroups of laboratories was tracked over time (1976 to 1980) when challenged with this type of sample as shown in Fig. 1.

The performance of subgroup A laboratories improved over time. In 1976, the percent error (failure to report *N. gonorrhoeae*) for subgroup A was about 27%; the percent error decreased (as shown in Fig. 1) to about 9% in 1980. For comparison, in 1976 the percent errors for subgroup D and subgroup E were about 5 and 1.6%, respectively. Except for the occurrence (1978 II shipment) in which the percent error for subgroup D was 12%, the percent error for this subgroup ranged from 2.5 to 8%. For subgroup E, it ranged from 0 to about 6% and was consistently lower than the other two subgroups.

The peak (1978 II shipment) shown in Fig. 1 for laboratory subgroup D and the peak (1977 II) for laboratory subgroup E were related to test results with mixture-type samples containing *N. gonorrhoeae, S. aureus*, and *S. faecalis*. This type of sample presented a particular problem to those laboratories that employed CTA media for CU tests in confirmatory testing. The erroneous results appear to have been related in part to the failure to use a pure culture of *N. gonorrhoeae* for inoculation of CTA media.

With reference to the VS *N. gonorrhoeae* strain, 22% of the subgroup A laboratories were unsuccessful in isolating/identifying the strain as compared with 13% for subgroup D; about 7% of the subgroup E laboratories (State Health Departments) were unsuccessful.

The performance of individual laboratories over time could also be tracked to identify those laboratories with chronic testing problems. As an example of this, in 1980 a laboratory was identified with a percent error of 61% (obtained incorrect results with 14 of 23 samples tested). About 30% of the errors were with the pure-type samples and 70% were with the mixture-type. Essentially, the problem of this particular labo-

TABLE 5. Beta-lactamase test results reported with four proficiency testing samples containing PP *N. gonorrhoeae*

Test method	No. positive/no. of tests (% positive) for sample:			
	AG7-AO5	AG7-BO6	AG7-BO7	AG0-AO5
Agar disk diffusion	90/98 (91.8)	100/107 (93.5)	87/103 (84.5)	89/105 (84.8)
Chemical tests				
Acidometric	33/34 (97.1)	31/34 (91.2)	28/32 (87.5)	41/45 (91.1)
Iodometric	18/19 (94.7)	26/29 (89.7)	22/24 (91.7)	32/33 (97.0)
Chromogenic cephalosporin	5/5 (100.0)	8/8 (100.0)	8/8 (100.0)	28/29 (96.6)

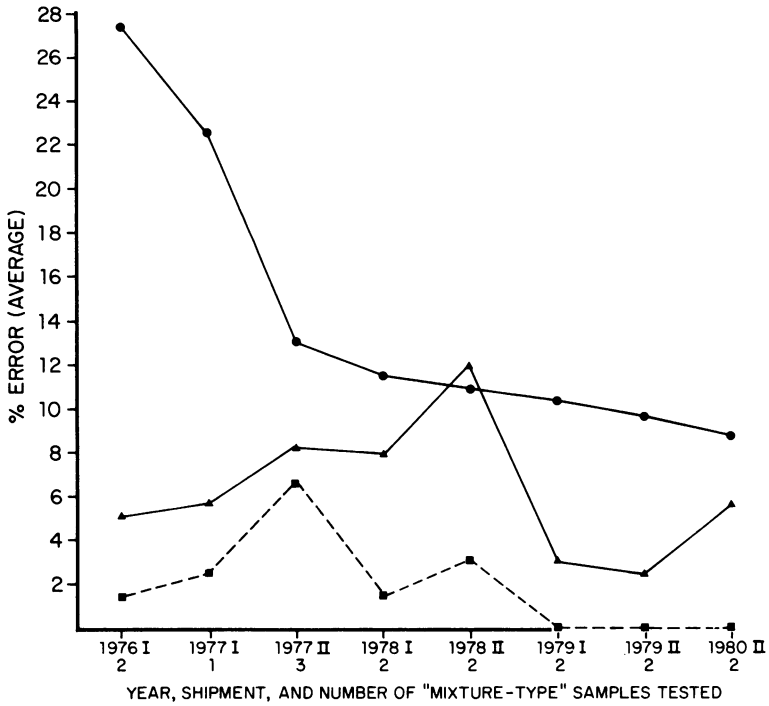

FIG. 1. Errors (%) by three laboratory subgroups by year and shipment with 16 samples containing *N. gonorrhoeae* and other microbial species. Symbols: ●, laboratory subgroup A (53 laboratories testing 0 to 12 patient specimens per day); ▲, laboratory subgroup D (70 laboratories testing >100 specimens per day; ■, laboratory subgroup E (37 State Health Department laboratories).

ratory was limited to the conduct of confirmatory CU tests; about half of the errors detected were due to negative glucose test results, and the other errors were due to positive maltose results; positive sucrose and lactose results were also reported in about half of the tests in which maltose results were positive.

DISCUSSION

The degree of success various laboratories have in isolating and identifying *N. gonorrhoeae* (as well as other microbial species) is dependent upon a number of factors, some of which are: (i) methods for collecting and handling specimens; (ii) quality of media and reagents; (iii) isolation and identification procedures; (iv) internal quality control procedures; (v) training and experience of laboratory personnel; and (vi) management and supervisory practices (22).

Internal quality control procedures, if adequately designed and applied, are usually more appropriate for detecting day-to-day problems. However, problems and errors that otherwise might not be identified can sometimes be detected through an external quality control program by interlaboratory comparisons. The problems identified by an external program are usually chronic, such as the use of an inadequate method or culture medium or the failure to use an established method correctly.

As presented above, the percentage of errors reported by laboratories doing presumptive testing only was significantly higher than that reported by laboratories performing confirmatory testing. Problems and errors were observed with initial procedures for cultivation, selection, and identification of gonococci. A small number (six) of laboratories reported the single use of chocolate agar for primary plating of the samples; as would be anticipated, the number of positive presumptive test results reported by these laboratories with samples containing *N. gonorrhoeae* mixed with other microbial species was lower than that reported by laboratories using a selective medium. As a minimum for primary plating, laboratories should use a selective medium such as one of the following: modified Thayer-Martin medium (13), improved Thayer-Martin (Martin-Lewis) medium (15), or New York City medium (7–9).

As a general statement, it is usually considered good practice to inoculate a nonselective medium in parallel with a selective medium for the initial plating of specimens. A selective medium represents a compromise in that the selective agent(s) used to suppress the growth of

undesired species may inhibit the growth of desired species, the degree of inhibition being dependent upon the strain, the population of cells in the inoculum, and the final concentration of the selective agent(s) in the medium. Also, a specimen may contain a clinically relevant microbial species whose growth is inhibited by the selective medium in use and whose presence was not suspected during examination of the specimen. Economics sometimes dictate the use of one medium (or the practice of inoculating several different specimens onto demarcated areas of one plate). About half of the reports received during 1979–1980 from laboratories in the CDC program listed the use of one plating medium; of these, about 95% listed the use of a selective medium.

With each mixture-type sample containing *N. gonorrhoeae*, some laboratories reported growth on a selective medium but did not obtain all positive presumptive findings. There are probably several explanations for such results, and one consideration is the quality of the medium or media used for primary plating. Despite improvements in the quality of media used for the isolation of *N. gonorrhoeae*, some problems have been experienced; it was reported (12) that about 14% of commercially prepared media used in 1979 for the isolation of *N. gonorrhoeae* were unsatisfactory. It is necessary and reasonable for laboratories to have quality control procedures for media (3), whether the media are purchased in ready-for-use form or are prepared within laboratories from purchased components (dehydrated media and additives). Media must also be stored according to the manufacturer's directions and discarded when outdated. A selective medium must be carefully prepared to obtain the selectivity required for its particular use and then stored properly to maintain this important characteristic.

In the instructions accompanying shipments of proficiency testing samples, laboratories were cautioned against holding restored freeze-dried samples before primary plating because of the possible overgrowth of *N. gonorrhoeae* by some species present in mixture-type samples. This consideration is, of course, important in handling many kinds of microbiological specimens containing more than one microbial species; prolonged holding under certain conditions may also result in a loss of microbial viability (10).

The failure to provide the incubation conditions necessary for the growth of gonococci may be another factor in determining whether gonococci were recovered from samples; proper moisture, atmospheric conditions, and temperatures must be provided (15).

Detection of the VS *N. gonorrhoeae* strain included in proficiency testing presented a problem to some laboratories. There have been several reports (4, 6, 17, 18, 23) of VS *N. gonorrhoeae* strains in the literature, and these strains may represent 3 to 10% of gonococcal strains. The challenge of detecting VS *N. gonorrhoeae* strains can be met partially by the use of a nonselective medium (such as chocolate agar) or by modifying the formula of a selective medium, for example, modified New York City medium (9), or both.

Other problems that led to negative presumptive results with samples containing *N. gonorrhoeae* may have been related to errors such as: (i) selection of colonies other than those of gonococci for examination; (ii) failure to obtain pure cultures of gonococci for further examination; (iii) failure to properly perform the oxidase test or use of an unsatisfactory oxidase test reagent; (iv) failure to properly perform the Gram stain procedure; (v) misinterpretation of microscopic observations; (vi) failure to apply quality control measures to media, procedures, and reagents; or (vii) clerical errors in reporting test results.

A few laboratories experienced problems with some negative-type samples that did not contain *Neisseria* species. It was anticipated that some laboratories performing only presumptive testing would experience problems with samples containing certain *Neisseria* species (such as *N. lactamica*) or *Moraxella* species. However, some laboratories reported *N. gonorrhoeae* for samples containing organisms such as *S. aureus*, *Alcaligenes faecalis*, *C. albicans*, *G. vaginalis*, and *L. monocytogenes*; these reports indicate problems with some very basic procedures and suggest a need for training of personnel.

A throat sample containing *N. meningitidis* and other species was sent to laboratories in the 1978 I shipment, but the report form in use at that time did not offer participants the option to report "*Neisseria*, species to be determined," after this shipment, this reporting option was added to the report form for samples designated as being from throat or anal sources. The use of the reporting option "*Neisseria*, species to be determined" was discussed in summary analysis reports sent to participating laboratories. However, we were not too successful in educating the participants using only presumptive tests to report "*Neisseria*, species to be determined" for *Neisseria* isolates contained in throat or anal samples. It has been recommended (16) that confirmatory tests be performed with isolates from urogenital sites, and confirmation is required for isolates from sources other than urogenital sites. Laboratories that perform presumptive testing can only report "*Neisseria*, species to be determined" for isolates from sites other than urogenital sites (11) until confirma-

tion is obtained (presumably from another laboratory to which such isolates are referred).

Significantly fewer errors were observed with reports from participants using confirmatory tests, and most of the errors in confirmatory testing were related to CU tests and to the use of CTA in particular. Pitfalls in the use of CTA for carbohydrate testing have been described (2, 11, 16, 19) together with methods for resolving problems in testing. It was apparent from data collected in the program that errors with CTA carbohydrate testing were more frequent with mixture-type samples than with pure-type samples. The frequency of errors was related to the number of fermenters present in mixture-type samples and indicated that the failure to obtain pure cultures of gonococci for inoculation of CTA was responsible in part for erroneous positive test results. This type of error was also apparent with other methods (rapid fermentation test and Minitek) for CU but not with other types of confirmatory tests (FA and CA).

Erroneous positive carbohydrate test results may indicate several problems: (i) failure to carefully select and pick colonies so that pure cultures are obtained, (ii) failure to follow recommended procedures, and (iii) failure to train personnel in the recommended procedures. With some procedures or circumstances, a purification plate is required to obtain a sufficient amount of growth of a pure culture (5, 11, 16) as inoculum in carbohydrate testing; the pitfalls of using growth from primary selective media as an inoculum should be recognized (16). The decision to bypass the purification plate step is often based on economics and time considerations. However, before making the decision to bypass the purification step, a laboratory should perform tests on each isolate with and without purification to determine whether bypassing the step introduces error into the confirmatory carbohydrate testing results.

The general perception that, as a group, the laboratories testing small numbers of patient specimens do not perform as well as laboratories testing larger numbers is supported by the data obtained from laboratories enrolled in this external program. However, it is not implied that all laboratories testing small numbers of specimens are not capable of producing results of acceptable quality. If a laboratory that tests small numbers of specimens cannot improve and maintain the quality of its test results, then its director should consider an arrangement with another, perhaps larger, laboratory to test the specimens or isolates from the specimens.

In spite of its limitations, an external quality control program offers the following opportunities and benefits: (i) different types of stable, freeze-dried samples can be sent for testing to laboratories with a high degree of confidence in the quality of the sample when received; (ii) individual laboratories and subgroups of laboratories experiencing difficulties can be identified by tracking their performance over time; (iii) once identified, assistance can be directed to those laboratories with problems; (iv) tests of difficulty and testing methods presenting chronic problems can be identified, and remedial measures can be proposed; (v) an external program can supplement individual laboratory internal quality control efforts and provide a vehicle for the continuing education of laboratory personnel; (vi) summaries of testing results allow individual laboratories to compare their performance and methods with those of other laboratories; (vii) methods and specific tests can be evaluated, allowing inadequacies to be identified that otherwise might not be detected; (viii) information on commonly used tests and new tests can be provided as a basis for choosing better or more standard methods, or both; (ix) the shifts in usage of different media and tests over time can be tracked; (x) a large base of test data can be developed for assessing the state of the art and state of the technology to evaluate the need for standard or reference methods.

Probably the two most important factors in the survival and continuation of external quality control programs, other than budgetary considerations, are their value (i) in interlaboratory comparisons and (ii) in the continuing education of laboratory personnel. It is incumbent upon external program providers to establish credibility in the program by providing stable testing samples of acceptable quality and by being responsive to problems identified in the testing of laboratories.

LITERATURE CITED

1. **Anhalt, J. P., L. D. Sabath, and A. L. Barry.** 1980. Special tests: bactericidal activity, activity of antimicrobics in combination, and detection of β-lactamase production, p. 483–484. *In* E. H. Lenette, A. Balows, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
2. **Arko, R. J., K. G. Finley-Price, K. Wong, S. R. Johnson, and G. Reising.** 1982. Identification of problem *Neisseria gonorrhoeae* cultures by standard and experimental tests. J. Clin. Microbiol. **15**:435–438.
3. **Blazevic, D. J., C. T. Hall, and M. E. Wilson.** 1976. Cumitech 3, Practical quality control procedures for the clinical microbiology laboratory. Coordinating ed., A. Balows. American Society for Microbiology, Washington, D.C.
4. **Brorson, J. E., J. Holmberg, B. Nygren, and S. Seeberg.** 1973. Vancomycin sensitive strains of *Neisseria gonorrhoeae*. A problem for the diagnostic laboratory. Br. J. Vener. Dis. **49**:452–453.
5. **Brown, W. J.** 1977. Stability of working reagents for the modified rapid fermentation test (MRFT). Health Lab. Sci. **14**:172–176.
6. **Cross, R. C., M. B. Hager, R. Neibaur, B. Pasternak, and F. J. Brady.** 1971. VCN-inhibited strains of *Neisseria gonorrhoeae*. HSMHA Health Rep. **86**:990–992.
7. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1978.

The selectivity of vancomycin and lincomycin in NYC medium for the recovery of *N. gonorrhoeae* from clinical specimens. Health Lab. Sci. **15**:22–27.

8. **Faur, Y. C., M. H. Weisburd, M. E. Wilson, and P. S. May.** 1973. A new medium for the isolation of pathogenic *Neisseria* (NYC medium). I. Formulation and comparisons with standard media. Health Lab. Sci. **10**:44–54.
9. **Granato, P. A., C. Schneible-Smith, and L. B. Weiner.** 1981. Use of New York City medium for improved recovery of *Neisseria gonorrhoeae* from clinical specimens. J. Clin. Microbiol. **13**:963–968.
10. **Isenberg, H. D., F. D. Schoenknecht, and A. vonGravenitz.** 1979. Cumitech 9, Collection and processing of bacteriological specimens. Coordinating ed., S. J. Rubin. American Society for Microbiology, Washington, D.C.
11. **Kellogg, D. S., Jr., K. K. Holmes, and G. A. Hill.** 1976. Cumitech 4, Laboratory diagnosis of gonorrhea. Coordinating ed., S. Marcus and J. C. Sherris. American Society for Microbiology, Washington, D.C.
12. **Lewis, J. L., and P. J. Weisner.** 1980. Gonorrhea: current laboratory methods. Lab. Manage. **18**:33–36; 43.
13. **Martin, J. E., J. H. Armstrong, and P. B. Smith.** 1974. New system for cultivation of *Neisseria gonorrhoeae*. Appl. Microbiol. **27**:802–805.
14. **Martin, J. E., and A. Lester.** 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Rep. **86**:30–33.
15. **Martin, J. E., and J. S. Lewis.** 1977. Anisomycin: improved antimycotic activity in modified Thayer-Martin medium. Public Health Lab. **35**:53–62.
16. **Morello, J. A., and M. Bohnhoff.** 1980. *Neisseria* and *Branhamella*, p. 111–130. *In* E. H. Lenette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
17. **Odegaard, K., O. Solberg, J. Lind, G. Meyhre, and B. Nyland.** 1975. Lincomycin in selective medium for the isolation of *Neisseria gonorrhoeae*. Acta Pathol. Microbiol. Scand. Sect. B. **83**:301–304.
18. **Reyn, A., and N. W. Bentyon.** 1972. Comparison of a selective and non-selective medium in the diagnosis of gonorrhoeae to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. Br. J. Vener. Dis. **48**:363–368.
19. **Sonnenwirth, A. C.** 1980. Gram-positive and gram-negative cocci, p. 1658–1659. *In* A. C. Sonnenwirth and L. Jarett (ed.), Gradwohl's clinical laboratory methods and diagnosis, 8th ed. The C. V. Mosby Company, St. Louis.
20. **Thayer, J. D., and J. E. Martin.** 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. Health Lab. Rep. **81**:559–562.
21. **Thornsberry, C., T. L. Gavan, and E. H. Gerlach.** 1977. Cumitech 6, New developments in antimicrobial susceptibility testing. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
22. **Wilcox, K. R., Jr., T. E. Baynes, Jr., J. V. Crable, J. K. Duckworth, R. H. Huffaker, R. E. Martin, W. L. Scott, M. V. Stevens, and M. Winstead.** 1978. Laboratory management, p. 3–126. *In* S. L. Inhorn (ed.), Quality assurance practices for health laboratories. American Public Health Association, Washington, D.C.
23. **Windall, J. J., M. H. Hall, J. H. Washington II, T. J. Douglas, and L. A. Weed.** 1980. Inhibitory effects of vancomycin on *Neisseria gonorrhoeae* in Thayer-Martin medium. J. Infect. Dis. **142**:775.