EVALUATION OF THE QUALITY OF BACTERIOLOGIC METHODS USED TO DIAGNOSE TUBERCULOSIS IN ARGENTINA[1]

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INTRODUCTION

More and more Latin American and Caribbean countries are integrating the activities of their national tuberculosis programs into the general health services. For this and other reasons, the number of such services doing bacteriologic diagnosis of tuberculosis, usually by sputum-smear microscopy, is increasing. In addition, some of the dispensaries, hospitals, and general services with laboratories of some complexity have added culture diagnosis to their work. A recent survey conducted in 12 Latin American countries *(1)* found that 2,884 laboratories at level III (laboratories with microscopy only) were employing only sputum microscopy to diagnose tuberculosis, while 456 others, at level II (laboratories with microscopy and culture facilities) and at level I (with microscopy, culture, and differentiation test facilities) were also using bacteriologic cultures.

The culture technique is a more sensitive diagnostic method than direct sputum microscopy. It has been determined that sputum microscopy stands only a 50% chance of yielding a positive result if the sample contains between 5,000 and 10,000 acid-fast bacilli. In contrast, the culture technique is virtually certain to yield at least one colony if 10 bacilli are present *(2)*. Thus, when correctly employed, the culture technique is a highly useful tool for diagnosing paucibacillary cases of tuberculosis—such as pulmonary forms with negative smear examinations, extrapulmonary forms, and pediatric cases. On the other hand, the method is more costly and technically complex than direct sputum microscopy.

The survey mentioned above *(1)* also reported that 48 laboratories in

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the 12 survey countries, in addition to performing sputum microscopy and culture diagnosis, tested the sensitivity of *Mycobacterium tuberculosis* to antibiotic agents and, in some cases, performed mycobacterial differentiation tests. Generalized performance of sensitivity tests is not advisable because of their technical complexity and currently limited utility—utility that is limited because adoption of treatment regimens with several antibacterial drugs has overcome the problem posed by the appearance of bacterial resistance *(3)*. Nevertheless, sensitivity testing is indicated for cases needing retreatment, for therapeutic failures, and for epidemiologic surveys of primary and acquired resistance.

Mycobacterial differentiation, which requires the right technical equipment and personnel with the bacteriologic experience and judgment needed to perform the work involved correctly, can help determine the frequency with which nontuberculous ("atypical") mycobacteria are being isolated in particular laboratories, as well as the relative importance of the different mycobacterial diseases and of bovine tuberculosis in man in areas where current information on these problems is scarce *(4, 5)*.

With respect to standards, PAHO/WHO has published standards for tuberculosis bacteriology tests that range from sputum-smear microscopy to tests for typing mycobacteria *(6, 7, 8)*. Also, in most countries of the Americas tuberculosis control programs have published national standards.

Regarding training, two regional reference laboratories give training courses and provide advice and services in the field of tuberculosis bacteriology. These are the Central Disease Control Laboratory (a WHO Collaborating Center) in Ottawa, Canada, and the Pan American Zoonoses Center (CEPANZO, PAHO/WHO) in Martínez, Argentina. In general, most countries' control programs enjoy the services of well-trained and experienced bacteriologists.

Given these circumstances, development of supervisory systems to control the methods used in tuberculosis bacteriology is both possible and essential. Such systems will permit evaluation and improvement, as needed, of the efficiency of diagnosis and bacteriologic control through treatment, as well as evaluation and improvement of the quality of epidemiologic information on tuberculosis and other mycobacterial diseases in Latin America and the Caribbean. For such reasons several countries already have programs for supervising the quality of sputum-smear microscopy *(9)*.

Turning specifically to Argentina, that country currently has 423 official laboratories (at level III) that do only sputum-smear microscopy, another 50 (at level II) that do both sputum microscopy and culture diagnosis, and 10 (at level I) whose services include mycobacterial differentiation *(1)*.

At present, the National Institute of Epidemiology (INE) in Santa Fe provides coordination at the national level for supervision of sputum-smear microscopy conducted by the central laboratories of the various provincial tuberculosis control programs *(10)*. With a view to extending this supervision to other bacteriologic techniques, in 1980 the Argentine Commission on Tuberculosis Bacteriology proposed a cooperative survey that might provide the basis for a stable quality-control program. This article presents the results of that survey.

Materials and Methods

Indirect Supervision of Sputum-Smear Microscopy

During 1985 the INE, in its role as supervisory coordinator, directed the central laboratories of the provincial tuberculosis control programs to submit slides from sputum-smear examinations that they had received during a single month from the laboratories under their supervision. This request was made each month to the central laboratories of three different provinces throughout the one-year 1985 study period.

Up to 100 of the slides received were reread at the INE. When more than a hundred slides were received from a given health service, all those that had yielded positive results were reread together with a random sample of the ones yielding negative results, up to the point where the number of reread positive and negative slides totaled 100.

Besides checking the results, the INE classified the quality of the sputum specimen, the smear, and the stain in accordance with procedures that have already been described *(11)*. That is, the sputum was classified as mucous, mucopurulent, or consisting mainly of saliva; the smear was classified as good, thin, thick, or uneven; and the staining was classified as good, good with comments, or inadequate. The quantitative reading was done according to national standards, which are the same as those recommended by PAHO/WHO *(6)*. The results were compared with those obtained by the peripheral laboratories and were communicated to them through the provincial control programs.

Evaluation of Culture Media Quality

In general, evaluation of the culture media being used for diagnosis provides useful insight into the relative sensitivity that bacteriologic diagnosis of tuberculosis can attain vis-a-vis the quality of the medium employed. In general, the less sensitive the medium the larger the number of bacilli needed in the sample in order to get a positive result. Inoculated on a medium of low sensitivity, for example, paucibacillary samples (such as those from cerebrospinal fluid) could give false negative results. Observations about the medium's presentation, appearance, consistency, and contamination can also help to improve the techniques used in its preparation.

Quality control of this kind makes it possible to know at the central level when a laboratory is using a medium of relatively low sensitivity, to review with it the methods being used for preparation and preservation, and to introduce any necessary changes. We believe that such quality control of culture media can be easily implemented on an ongoing basis in the central tuberculosis laboratories of those Latin American and Caribbean countries where the culture technique is being used.

With regard to the study reported here, each of the 13 participating laboratories sent the Center for Tuberculosis and Pulmonary Diseases *(Cãtedra de Tisioneumonología)* a sample from a batch of recently prepared Lowenstein-Jensen medium, together with the data requested in Form 1 (Annex 1). The 13

batches were tested in a single experiment, which was conducted according to a protocol prepared at CEPANZO. The strain used was *M. tuberculosis* H37Rv, from which suspensions were prepared at 10^{-4} mg/ml, 10^{-5} mg/ml, and 10^{-6} mg/ml—starting with a bacillary suspension of 1 mg (wet weight) per ml. The next step was to inoculate 0.2 ml from each of these three bacillary dilutions into four tubes of the medium being tested. These were incubated at the usual temperature of 37°C *(7)*, and the resulting colonies were counted at three, four, and five weeks. All of the tubes were coded so that those doing the work did not know the source of the test batch—either at the time of inoculation or when the number of colonies was read.

It would be possible for the number of colonies appearing in each of the four tubes inoculated with the same dilution to exhibit normal variation within certain limits. This variation was evaluated by the chi-square test. If χ^2 exceeded the critical value for the number of tubes (n = 4)—namely, 7.8 (P = 0.05) *(12)*—it would be taken as an indication of significant experimental error beyond the normal variation in inoculum from one tube to another; and so the experiment would need to be repeated.

The indicators of quality for each of the controlled batches were: (a) the total number of colonies in the 12 tubes containing the test batch; (b) the relationship between the number of colonies observed at three and at five weeks; and (c) the number of colonies in the tubes inoculated with the 10^{-5} suspension, this being the lowest bacillary concentration yielding growth in all of the controlled batches.

It was decided that a batch would be deemed to have significantly low sensitivity if the total number of colonies obtained was less than the average value minus two standard deviations ($\bar{X}$ − 2 SD).

Antibiotic Sensitivity Test Evaluation

Five laboratories sent 299 *M. tuberculosis* cultures tested for isoniazid and streptomycin sensitivity to the Regional Tuberculosis Laboratory in Córdoba, where the sensitivity tests were repeated. The results were then compared with those obtained by the laboratory of origin. In all cases the technique employed was the low-cost variant of the previously described proportions method using Lowenstein-Jensen medium *(8)*.

Evaluation of Mycobacterial Differentiation

Fifteen laboratories participated in this part of the study, which was conducted over a three-year period (January 1982 through December 1984). Each of them sent the National Institute of Microbiology (INM) all the cultures that had yielded isolates of mycobacteria and that, according to their own judgment, had at least one of the following characteristics: (a) growth in Stonebrink medium but little or no growth in Lowenstein-Jensen medium; (b) abundant growth in both these media within less than 10 days; (c) morphology or pigmentation of the colonies different from that of *M. tuberculosis;* (d) resistance to several drugs in the case of cultures obtained from previously untreated patients; (e) a negative niacin test.

Accompanying each culture was the following information: (a) the type of sample (sputum, urine, etc.); (b) the number of positive cultures previ-

ously obtained from the same patient; (c) the number of colonies grown in each of these cultures; (d) the patient's name, place of residence, and other data of interest; and (e) the treatment given.

Final identification of the mycobacteria in the cultures received was accomplished by the INM with the support of CEPANZO, using previously described methods *(8, 13)*.

Results

Sputum-Smear Microscopy

A total of 2,293 slides from 316 laboratories in 14 provinces were received and checked at the INE. The INE readings indicated that 66.8% of these came from adequate samples classified as either mucous or mucopurulent. The remaining 33.2% were classified as consisting mainly of saliva, either because the sample was inadequate or because an incorrect portion of it had been selected.

As the proportion of defective samples increases, the probability of finding bacilli in smears from tuberculosis patients tends to decline. Table 1 shows the share of positive findings in the present study accounted for by samples in the three aforementioned categories.

TABLE 1. Positive sputum-smear examinations obtained with sputum samples classified as consisting of saliva or as being mucous or mucopurulent (Argentina, 1985).

Classification of sample	No. of positive samples	% of all positive samples
Saliva	34	11.4
Mucous	75	25.1
Mucopurulent	190	63.5
Total	299	100

In 97% of the slides the staining was classified as good because 100 microscopic fields could be read that were stained and did not contain fuchsin crystals. Table 2 shows the differences found between the INE readings and those taken at the laboratories of origin, according to the quality of the staining. The proportion of discrepancies found in the slides with deficient staining (7.5%) was 4.5 times greater than that found in the slides with good staining (1.6%). All of the discrepancies found in the inadequately stained slides involved false positive results.

Table 3 summarizes the results obtained by comparing all the readings from the 14 participating provinces with the INE rereadings. Agreement between these two sets of readings was 98.0%, within a range of 92.6–100.0%. Of 1,994 slides read as negative at the INE, 24 (1.2%) were reported as positive by the laboratories of origin. On the other hand, 22 of the 299 slides whose INE readings were positive (7.4%) had been considered negative by the laboratories of origin.

As in a previous survey *(10)*, the rate of disagreement was very high in the case of slides that had few bacilli. Specifically, five of the eight slides (62.5%) in which the first observer had found only 1-4 acid-fast bacilli (AFB) in the entire slide were read as negative at the INE. However, the disagreement lessened as the level of slide positivity increased. That is, there was 7.5% disagreement for slides classified (+) by the laboratory of origin, 4.7% for those classified (+ +), and 0% for those classified (+ + +).

TABLE 2. Quality of sputum-smear staining and disagreements between originating laboratory and INE readings (Argentina, 1985).

Classification of staining quality	No. of smears	Disagreements		Number of	
		No.	(%)	False positives[a]	False negatives[a]
Inadequate	67	5	(7.5)	5	0
Good with comments	423	12	(2.8)	7	5
Good	1,803	29	(1.6)	12	17
Total	2,293	46	(2.0)	24	22

[a]Based on the assumption that the INE readings were correct.

TABLE 3. Results of quality control of sputum-smear examinations comparing original readings in the 14 participating provinces with the INE rereadings (Argentina, 1985).

		Central laboratory (INE) Result		
	Result	+	−	Total
Peripheral laboratories	+	277	24	301
	−	22	1,970	1,992
	Total	299	1,994	2,293

Agreement of results: 2,247 (98.0%)
Disagreement of results: 46 (2.0%)
False positives (assuming INE result correct): 24 (1 2%)
False negatives " " " " . 22 (7.4%)

Culture Media

Table 4 shows the number of colonies grown on a normal batch of controlled Lowenstein-Jensen medium in 12 tubes receiving different concentrations of inoculum. Chi-square analysis did not reveal any significant variations in the numbers of colonies observed within different tubes receiving the same inoculum concentration.

Table 5 presents the results obtained from similar tests run on 13 batches of media from the 13 participating laboratories. The smallest number of colonies observed in any one group of 12 tubes at five weeks was 159. This number is higher than the value considered critical ($\bar{X}$ − 2 standard deviations = 279 − (2 × 67) = 145).

The length of time needed to grow the colonies was deemed appropriate for *M. tuberculosis* in this culture medium—especially considering that the number of colonies found in readings taken after three weeks of incubation was between 84% and 100% of the number found after five weeks.

Another indicator of the media's sensitivity was the number of colonies observed in the tubes inoculated with 0.2 ml of the 10^{-5} mg/ml solution—this being the weakest inoculum that yielded growth in all of the 13 batches tested. The ratio of the minimum to the maximum number of colonies grown in the 13 batches was 1:6 (14:78 colonies). Correlations were found between all three of the indicators listed in Table 5, but especially between the total number of colonies observed and the number observed in tubes receiving the 10^{-5} dilution.

TABLE 4. Culture results obtained with a normal batch of Lowenstein-Jensen medium (batch No. 842) that was inoculated on 12 April 1986 with 0.2 ml of the bacterial suspensions shown, incubated at 37°C for five weeks, and read on 17 May.

Concentration of bacterial inoculum (mg/ml)	No. of colonies grown in each of four tubes	Total no. of colonies	Average no. of colonies ($\bar{X}$)	Chi-square[a]
10^{-4}	65 70 75 80	290	72.50	1.7
10^{-5}	14 15 17 10	56	14.00	1.9
10^{-6}	1 1 2 2	6	0.75	3.7

[a] $X^2 = n S x^2/Sx - Sx$, where n = the number of tubes for each dilution (four in this case) and S = "the sum of."

TABLE 5. Results of quality control of Lowenstein-Jensen media from 13 laboratories showing the numbers of colonies grown in all 12 tubes (see Table 4), the percentage of colonies found at three (as compared to five) weeks of incubation at 37°C, and the numbers of colonies grown in the four tubes inoculated with 0.2 ml of the bacterial suspension at a concentration of 10^{-5} mg/ml.

Batch No.[a]	Total No. of colonies grown[b]	% colonies found at 3 (as compared to 5) weeks of incubation	Total No. of colonies grown on 4 tubes receiving 10^{-5} mg/ml inoculum
1	382	96.9	78
2	353	95.8	70
3	352	96.9	56
4	336	89.6	48
5	311	91.3	38
6	305	88.9	46
7	281	99.3	24
8	250	86.0	23
9	241	84.2	21
10	225	85.8	24
11	224	97.8	23
12	209	95.7	26
13	159	84.9	14
	Average ($\bar{X}$):279	Over 90%:7	Average ($\bar{X}$):37.8
	Standard deviation: ±67	Under 90%:6	Range:14–78

[a] Numbered from one to 13 in decreasing order of the number of colonies observed.
[b] Total observed at five weeks in 12 tubes, four inoculated with 0.2 ml of the 10^{-4} mg/ml bacterial suspension, four with 0.2 ml of the 10^{-5} mg/ml suspension, and four with 0.2 ml of the 10^{-6} mg/ml suspension.

Antibiotic Sensitivity Tests

Table 6 presents the results of retesting 299 cultures previously tested for sensitivity to isoniazid (INH) and streptomycin (SM) by five different laboratories. The retesting was performed at the Córdoba Regional Laboratory. Overall, there was 95.7% agreement between the two sets of results. Most of the disagreements related to SM resistance, the bulk of these involving an initial finding of resistance to that drug.

TABLE 6. Agreement between antibiotic sensitivity tests (to isoniazid and streptomycin) performed on 299 cultures by five laboratories and subsequent tests performed on the same cultures by the Córdoba Regional Laboratory in 1986.

Laboratory No.	No. of tests performed	Agreement		False[a] sensitivity to:		False[a] resistance to:	
		No.	(%)	Isoniazid	Streptomycin	Isoniazid	Streptomycin
1	62	60	(96.8)	–	2	–	–
2	93	92	(98.9)	1	–	–	–
3	49	44	(89.8)	–	2	1	2
4	17	15	(88.2)	–	–	–	2
5	78	75	(96.2)	–	–	–	3
Total	299	286	(95.7)	1	4	1	7

[a]Based on the assumption that the regional laboratory results were correct.

Mycobacterial Differentiation

During the 1982–1984 study period, the 15 participating laboratories obtained 13,544 cultures identified as mycobacteria, of which 602 had been designated as possibly "atypical" mycobacteria and the remainder as *M. tuberculosis.* The 602 "atypical" cultures were sent to the National Institute of Microbiology (INM) with the corresponding information. There it was found that six of them (1%) did not belong to the genus *Mycobacterium* while 123 of them (20.4%) were actually strains of *M. tuberculosis.*

Table 7 shows the relationship between use of the niacin test by the 15 participating laboratories and correct differentiation between *M. tuberculosis* and other mycobacteria as indicated by retesting. Of the 95 cultures described as possibly "atypical" mycobacteria by the seven laboratories that did not use the niacin test, 38 (40%) were subsequently identified as *M. tuberculosis* by the INM. In contrast, only 16.8% of the 507 strains described as possibly "atypical" by the eight laboratories that did use the niacin test were subsequently identified as *M. tuberculosis.* It should also be noted that these eight laboratories using the niacin test appeared to have a much

TABLE 7. Correlation between use of the niacin test by 15 participating laboratories and accurate differentiation of *M. tuberculosis* from other mycobacterial strains, assuming the results obtained at the National Institute of Microbiology were correct.

Niacin test used	No. of laboratories	No. of positive cultures	No. of "atypical" cultures retested		
				Identified on retesting as *M. tuberculosis*	
			Total	No.	(%)
No	7	541	95	38	(40.0)
Yes	8	13,003	507	85	(16.8)
Total	15	13,544	602	123	(20.4)

TABLE 8. Evaluation by the INM Central Laboratory of mycobacterial identification tests performed by the INE level I laboratory participating in the study.

Taxonomic level reported by:		Cultures tested		Agreements		Disagreements	
The central laboratory	The participating laboratory	No.	(%)	No.	(%)	No.	(%)
Runyon group	Species	1	(1)	0	(0)	1	(1)
Species complex	Runyon group	12	(8)	10	(7)	2	(1)
Species complex	Species complex	20	(14)	17	(12)	3	(2)
Species	Runyon group	79	(56)	76	(54)	3	(2)
Species	Species	30	(21)	29	(20)	1	(1)
Total		142	(100)	132	(93)	10	(7)

larger volume of work, since they accounted for 96% of all the cultures studied.

Of all the participating laboratories, only one (the INE laboratory) performed biochemical tests—such as nitrate reduction, catalase activity (room at 58°C), tween 80 hydrolysis, growth on MacConkey agar, and pigment studies—in addition to the niacin test. Of the 142 strains selected by it during the abovementioned study period and sent to the INM, 91 had been classified as belonging to one of the Runyon groups; 20 others had been identified as belonging to a species complex (e.g., the *M. avium-intracellulare* complex, the *M. terrae* complex, etc.); and 31 had been tentatively identified by species, taking into account the technical limitations and probable clinical interest involved.

Table 8 shows the degree of agreement between the results obtained by the one participating laboratory (INE) and the INM central laboratory. Overall, there was 93% agreement between the two sets of results.

DISCUSSION AND CONCLUSIONS

The indirect supervision of sputum-smear microscopy indicated that the laboratories involved were applying the technique uniformly, undoubtedly in part because of broad dissemination of bacteriologic standards and effective training of microscopists throughout the country. The percentage of samples classified as consisting mainly of saliva could be reduced by giving patients more precise instructions about how to collect specimens.

The overall quality of the staining was good. The few slides with deficient staining tended to give false positive readings because fuchsin crystals were confused with bacilli.

The reproducibility of readings by the peripheral laboratories, as indicated by those made at the reference laboratory, was very good (98.0%). This finding is consistent with the reported results of a previous study *(10)*. Disparate results were more frequent with sputum smears that contained relatively few bacilli. Despite the generally favorable nature of the findings, these results underscore the desirability of having an ongoing quality control program for sputum-smear microscopy.

Regarding evaluation of the media being used to culture specimens, the 13 batches of media evaluated yielded a minimum of 159 and a maximum of 382 colonies, so that the maximum was no greater than 2.4 times the minimum. In this same vein, every batch yielded a total number of colonies exceeding the critical number of 145 ($\bar{X}$ − 2 SD). Hence, the results indicate that the general quality of media being used in the 13 participating laboratories was adequate.

However, on the basis of this first quality control trial of culture diagnosis, CEPANZO has modified the recommended bacillary concentrations of the inoculum and the recommended number of tubes to be employed as spelled out in the original protocol. The current protocol, as amended, calls for dilutions of 1×10^{-4} mg/ml and 0.5×10^{-4} mg/ml, a minimum of four tubes being inoculated with 0.2 ml each of the first dilution and twice as many (at least eight) being inoculated with the second in order to obtain less of a range in the readings and less likelihood of negative cultures *(14, 15)*.

Evaluation of antibiotic sensitivity testing at five laboratories demonstrated that the quality of testing in those laboratories was good. However, tests of streptomycin sensitivity were less reliable than those of isoniazid sensitivity. This appears to have been due mainly to the inferior thermal stability of dihydrostreptomycin sulfate, a conclusion underscoring the fact that maintaining proper storage conditions—both for the pure drug and for the medium once it is prepared—are of fundamental importance *(8)*.

With regard to mycobacterial differentiation, the results suggest it would be desirable to extend use of the niacin test to all level II laboratories that perform culture diagnosis. In addition, it appears important to upgrade knowledge of basic mycobacterial differentiation in these level II laboratories through specific training, so that the bacteriologists involved will know how to proceed in the event of unusual isolations. Overall, the results obtained in this study showed a high level of agreement between the two laboratories that performed tests identifying mycobacterial species or groups of species.

Summary

Bacteriologic methods—including sputum-smear microscopy, culturing, drug sensitivity tests, and tests distinguishing between different kinds of mycobacteria—are being used increasingly in Argentina and other Latin American and Caribbean countries to diagnose tuberculosis. This has created a growing need for adequate quality control systems to monitor and supervise performance of these tests.

To that end, in 1985 a cooperative quality control study of all such diagnostic methods was undertaken in Argentina. A total of 2,293 sputum smears from 316 laboratories were reread at Argentina's National Institute of Epidemiology in Santa Fe, and the quality of each specimen was assessed. Batches of Lowenstein-Jensen culture medium from 13 laboratories were tested by inoculation and culturing of *Mycobacterium tuberculosis* strain H37Rv at the University of Buenos Aires' Center for Tuberculosis and Pulmonary Diseases. *M. tuberculosis* cultures tested for sensitivity to streptomycin and isoniazid at five laboratories

were retested at the Regional Tuberculosis Laboratory in Córdoba. A total of 602 cultures of possibly "atypical" mycobacteria from 15 laboratories were evaluated at the National Institute of Microbiology in Buenos Aires. And 142 cultures of atypical mycobacteria classified at the National Institute of Epidemiology were retested and reclassified at the National Institute of Microbiology.

In general, the results of this extensive evaluation were satisfactory. There was 98% agreement between the original and subsequent sputum-smear readings, and the laboratories involved appeared to be applying the sputum-smear microscopy technique uniformly. The general quality of the culture media tested appeared adequate. The quality of antibiotic sensitivity testing was found to be good. And a high level of agreement was found between the two laboratories performing tests to classify atypical mycobacteria. Regarding mycobacterial differentiation between cultures of *M. tuberculosis* and "atypical" mycobacteria, the results suggest it would be desirable to have the niacin test used by all level II laboratories performing culture diagnosis.

Overall, it is felt that the procedures and methods used to perform these evaluations can serve as a useful reference model in Argentina and other countries for establishing a complete quality control system operating on a regular basis to monitor and supervise the tuberculosis laboratory network.

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ANNEX 1

Form No. 1: Quality Control of Tuberculosis Culture, Cooperative Interlaboratory Study

SUBMISSION OF CULTURE MEDIUM

Participating laboratory:

Date of shipment:

Lot no.:

Date of preparation:

No. of tubes in lot:

METHOD OF PREPARATION

Was a dehydrated commercial medium used?
YES NO

Brand:

Time and temperature of coagulation:

Was it tested for sterility? YES NO

If so, what type of test was used?