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Implementation of proficiency testing in conjunction with a rechecking system for external quality assurance in tuberculosis laboratories in Mexico

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SUMMARY

SETTING: In developing countries, tuberculosis is diagnosed by identification of acid-fast bacilli (AFB) on sputum smears.

OBJECTIVE: To evaluate the quality of AFB microscopy, the Mexican Secretary of Health National Reference Laboratory implemented proficiency testing for its network of 637 laboratories.

DESIGN: A total of 586 (92%) laboratories were inspected and 430 technicians evaluated by proficiency testing consisting of 10 slides with known numbers of AFB. Results were compared with those of slide rechecking and with proficiency testing performed 2 years later.

RESULTS: Of the 430 technicians evaluated by proficiency testing in 1998, 196 (46%) scored less than 80% and received intensive training in 1999. From a previous mean score of 65% their results increased to 90% ($P < 0.0001$). In 2001, they again underwent proficiency testing, and the mean score was 83%. The main factors affecting proficiency testing results were the type of laboratory in which the microscopists worked and the number of low-positive slides (1–9/100) in the test. Laboratories whose work was rechecked had better scores ($P = 0.002$). Proficiency testing scores and the estimated sensitivity of the microscopist's laboratory were associated ($P = 0.01$).

CONCLUSION: External quality assessment and training improve diagnostic performance. Rechecking and proficiency testing are both viable measures of laboratory performance.

KEY WORDS: external quality assurance; laboratory network; proficiency testing; rechecking AFB; tuberculosis

TUBERCULOSIS (TB) remains an important health problem worldwide, with more than 8 million cases estimated annually.[1] In Mexico, incidence rates range from 4 to 37 cases per 100 000 population, with around 23 000 new cases of TB diagnosed every year, 80% of which are pulmonary TB.[2] The most commonly used procedure for TB diagnosis is sputum smear examination of acid-fast bacilli (AFB).[3] The Mexican Secretary of Health's TB laboratory network provides diagnoses to around 40% of the population (39 million people), and AFB smears constitute 75% of the diagnostic tests used for tuberculosis.[4] It is therefore important to assure the quality of this diagnosis. The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) recommends several external quality assurance (EQA) methods for laboratory networks that support national control programmes, such as rechecking, which consists of reading a random selection of routine slides.[3,5] Alternatively, proficiency testing has been proposed, which consists of staining and reading centrally prepared slides with known numbers of AFB.[3,5]

Rechecking slides directly evaluates routine diagnosis, but it is very labour intensive. Due to a lack of coordination or resources, and because rechecking of routine smears is difficult to organise, many countries that rely on AFB smear microscopy for the diagnosis of TB have not implemented EQA, leading to a large number of false results.[6] Alternatively, proficiency testing consists of a reference laboratory producing slides with defined numbers of AFB that are checked by microscopists. Their performance is then assessed in the reference laboratory.[6] The Mycobacteria Department of the Institute for Epidemiological Diagnosis and Reference (InDRE) in Mexico recently conducted proficiency testing in conjunction with an inspection of the laboratories in the National TB Laboratory Network that perform AFB microscopy, to gather information about the quality of the laboratories. Rechecking is the

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routine quality control method that has been used in Mexico for many years. The results of proficiency testing were analysed along with the results of routine rechecking, and are presented in this paper.

MATERIALS AND METHODS

In order to implement proficiency testing (PT), the first step was to train two staff members from InDRE in the preparation of slides with defined numbers of AFB. The training was conducted at the State Public Health Laboratory (SPHL) of Nuevo Leon in collaboration with the Texas Department of Health (TDH) and the Centers for Disease Control and Prevention (CDC).

At InDRE, the newly trained staff prepared slides with 1–9 bacilli per 100 fields (low-positive), 1+, 2+ and negatives. For this purpose at least 5 ml of watery sputum samples without blood were obtained from TB patients (1+ or 2+). Negative AFB specimens were obtained from non-tuberculous patients with upper tract respiratory diseases and ⩾20 white blood cells. One drop of 40% formaldehyde was added per ml of sputum, mixed well and incubated for 1 h at room temperature; 1 ml of 4% NaOH was added and mixed for 5 min. Distilled water was added to 20 ml, mixed well and incubated at 55–60°C for 1 h for positive samples and for 10 min for negative samples. Distilled water was added to a total volume of 40 ml, mixed by inversion and centrifuged at 3000 × g for 20 min. Supernatants were discarded carefully and pellets were resuspended in 5 ml distilled water, and considered the stock solutions.

In order to obtain low positive, 1+ and 2+ samples, the stock solution of positive AFB sputum was diluted with the negative stock solution. For calculation of the dilution factor, the following formula was used: $N = (DC/AC) \times A$, where N is the amount of drops of positive sputum to be added, DC is the desired AFB concentration, AC is the actual AFB concentration and A is the number of drops in a given volume. To know the number of drops per ml, a Pasteur pipette may be used. AC was obtained in a smear made with two drops of the positive stock solution.

Finally, each smear was prepared with two drops of each final solution (low-positive, 1+ and 2+). Each sample was used to prepare 120–150 slides. Slide-to-slide consistency of AFB counts was validated by screening a minimum of six randomly selected slides per sample. Each PT slide set consisted of 10 stained slides classified using standard IUATLD and WHO[5] criteria for quantifying AFB, namely: no AFB seen, exact count (1–9 bacilli/100 fields), 1+ (10–99 bacilli/100 fields) and 2+ (100–999 bacilli/100 fields). A total of 350 slide sets were prepared. The grading system consisted of 10 points for each slide correctly identified as positive or negative, with two points deducted for each incremental difference in positive categories.

Fifteen microscopists from InDRE underwent a 1-week training course on the National Tuberculosis Programme prior to visiting and evaluating 586 of 637 laboratories in all 32 states and the Federal District (DF) of Mexico. The evaluators gave the PT set to the persons responsible for performing AFB microscopy, and used a standardised form to collect the following laboratory information: laboratory type, professional background of the laboratory employee, reagents, materials and equipment used, and quality of the microscopes.

To analyse the rechecking data in 1998, of the 283 002 AFB slides produced in 438 laboratories participating in the EQA rechecking programme, all positive and 10% of negative slides, randomly selected as suggested by the WHO, were sent from local laboratories for quality control to the SPHL,[6] where the diagnosis was usually known by the quality controller. Twenty-eight states had at least one local laboratory with complete rechecking data produced by an average of two controllers working at the SPHL; 303 laboratories (69%) had complete data, of which only 109 (36%) reported any errors. Information was sent to InDRE in standard format on the total number of positive and negative slides, the total numbers of positive and negative slides rechecked, error counts, and the specific types of errors; these data were used for further analysis.

In routine work, 10% of positive and 10% of negative slides are sent to InDRE, where rechecking is again performed and discrepancies between local laboratories and SPHL are identified, and if needed after restaining, slides are sent back to SPHL. Types of error were defined according to the IUATLD.[3] SAS software[7] general linear model techniques were used to identify laboratory factors associated with PT scores and rechecking sensitivity and specificity. The PT score for each individual was also compared with the sensitivity and specificity derived from the rechecking data for the laboratory where the individual worked. Confounder factors were analysed by a linear regression model using STATA.[7]

In 1999, those microscopists with scores <80% received 3 days of intensive training at the InDRE Department of Mycobacteria and underwent a second PT test immediately afterwards. In 2001, PT tests were sent by courier to all microscopists, who returned the slides with their scores. For the first PT in 1998, the distribution of slides with different AFB numbers was random in each set. In 1999 and 2001, the following were used: three negative slides, two with exact count, three graded 1+ and two graded 2+ per set. In 1998 and 1999, the proficiency testing of microscopists lasted 2 hours and was supervised. The same recommendation was given when the PT sets were sent to the laboratories, but duration could not be confirmed. PT data and low and high false-positive and false-negative results, as well as quantification

errors obtained in 1998 vs. 2001 were analyzed by Student's *t* test, paired Student's *t* test and χ^2 tests.

RESULTS

Mexico's Secretary of Health has a network of 637 laboratories where AFB microscopy is performed. PT was used to evaluate the accuracy of diagnosis among 604 microscopists from all 32 states and the DF. Results are presented for the 430 individuals who performed PT in 1998 and 2001 and who read five or more slides. This decision was taken because not all microscopists read all 10 slides in the set, and, to estimate properly the usefulness of proficiency testing for the evaluation of diagnostic capabilities, WHO indicates that in order to maintain diagnostic efficiency, each technician should read at least three slides per day.[5]

In 1998, 54% of microscopists had good scores (≥80%), 33% had marginal scores (60–79%) and 13% had poor scores (<60%). In 1999, 196 microscopists with scores <80% received intensive training and a second PT test immediately after. There was a significant mean increase in the scores of those who received training, from 65% to 90% ($P < 0.0000$); the overall average score was 78.8% in 1998 and 83.5% in 2001 ($P = 0.0000$). The factor most closely associated with PT score was the difficulty of the test, as measured by the number of low-positive slides in the set ($P = 0.0004$) (Table 1). In this case the analysis was based on the results of the 548 microscopists who underwent PT (including 118 who did so only in 1998). As the number of low-positive slides was associated with the difficulty of the test, subsequent sets had defined numbers of slides, i.e., three negative, two low-positive, three graded as 1+ and two as 2+.

The PT results of individuals working in different types of laboratories and with different degrees are shown in Table 2. In Mexico, individuals with approximately 5 years of post-secondary education in chemistry, microbiology and related subjects leading to a degree are called chemists. Laboratory technicians, on the other hand, generally receive approximately 2 years of post-secondary technical education. Statistically significant differences were found only in the comparison made with the 2001 data.

Fifty-nine laboratory quality factors were recorded:

Table 1 Mean scores for proficiency testing (PT) by number of low-positives for 548 microscopists in 1998

Number of low positives	Mean PT score (%)	Number of microscopists tested
0	86.3	36
1	82.0	104
2	80.6	373
3	74.9	30
4	76.0	5

Table 2 Mean scores for proficiency testing (PT) by laboratory type and professional degree for 430 microscopists

Laboratory type	Number of microscopists tested	Mean PT score	
		1998 (%)	2001 (%)
SPHL	34	76.7	90.7
Hospital	208	78.0	84.9
Health centre	306	79.5	82.2
Degree			
Chemist	218	78.2	80.5
Laboratory technician	166	80.4	83.1

SPHL = State Public Health Laboratory.

laboratories that had at least one microscope exclusively for AFB microscopy, a fluorescent microscope and water baths as well as repair programmes and performed AFB culture were found to be significantly associated with PT score. Non-significant quality factors included the number of different types of equipment present in the laboratory and preventive repair programmes, sufficient reagent supply, microscope and lenses in good working order, microscope not used exclusively for AFB diagnosis, good AFB staining technique and adequate working area.

To ensure the validity of these results, a multiple regression analysis was performed for confounders. The statistically significant confounders detected in 1998 were the total number of slides read by microscopists ($P = 0.003$) and the number of 1+ and 2+ slides ($P = 0.012$), while those found in 2001 were these two confounders ($P = 0.000$, $P = 0.024$, respectively) plus the type of laboratory ($P = 0.044$). R-squared values of these models were 0.09 and 0.23 for 1998 and 2001, respectively.

Microscopists in laboratories whose work was routinely rechecked had a better mean PT score than those in laboratories that were not rechecked (79% and 74%, respectively; $P = 0.002$). An initial comparison of PT and rechecking data did not reveal a significant association, as most of the laboratories reported no errors and there were large variations in the difficulty levels of the various slide sets. When the analysis was restricted to the 133 participants who received the most common slide set, consisting of five strong positives, two low positives and three nega-

Table 3 Agreement of the number of slides from local laboratories rechecked at the SPHL

	State Public Health Laboratory		
	Positive slides	Negative slides	Total slides
Local laboratory			
Positive slides	9569	81	9 650
Negative slides	193	29 029	29 222
Total slides	9762	29 110	38 872

SPHL = State Public Health Laboratory.

Table 4 False results in proficiency testing (PT) performed by 430 microscopists in 1998 and 2001

	False negative		False positive		Quantification errors	Concordant slides	Total slides
	High *n* (%)	Low *n* (%)	High *n* (%)	Low *n* (%)	*n* (%)	*n* (%)	
a) Results by year							
Year							
1998	205 (4.8)	311 (7.4)	77 (1.8)	43 (1.0)	745 (17.6)	2848 (67.4)	4229
2001	172 (4.1)	245 (5.7)	41 (0.9)	103 (2.4)	526 (12.3)	3182 (74.5)	4269
b) Results by laboratory type							
Laboratory type							
1998							
SPHL	15 (5.7)	19 (7.2)	4 (1.5)	1 (0.4)	55 (20.7)	171 (64.5)	265
Hospital	80 (5.2)	112 (7.3)	28 (1.8)	17 (1.1)	270 (17.6)	1027 (66.9)	1534
Health centre	110 (4.5)	180 (7.4)	45 (1.8)	25 (1.1)	420 (17.3)	1650 (67.9)	2430
2001							
SPHL	3 (1.1)	6 (2.2)	3 (1.1)	10 (3.7)	29 (11.9)	215 (80.8)	266
Hospital	55 (3.6)	94 (6.1)	12 (0.8)	31 (2.0)	187 (12.2)	1162 (75.7)	1536
Health centre	114 (4.6)	150 (6.1)	26 (1.0)	62 (2.5)	310 (12.6)	1805 (73.2)	2467
c) Results by qualification							
Degree							
1998							
Chemist	153 (5.1)	227 (7.5)	66 (2.2)	25 (0.8)	527 (17.5)	2011 (66.9)	3009
Laboratory technician	52 (4.3)	84 (6.9)	11 (0.9)	18 (1.5)	218 (17.9)	837 (68.6)	1220
2001							
Chemist	122 (4.0)	181 (5.9)	31 (1.0)	75 (2.5)	381 (12.4)	2271 (74.2)	3061
Laboratory technician	50 (4.1)	64 (5.3)	10 (0.8)	28 (2.3)	145 (12.0)	911 (75.5)	1208

SPHL = State Public Health Laboratory.

tives, from the 109 laboratories that had at least one error reported, the analysis revealed a significant association between PT results and sensitivity in rechecking smears ($P = 0.01$). This suggests that PT scores may be used to predict the sensitivity of AFB microscopy in a laboratory. Table 3 shows overall rechecking data used to evaluate sensitivity, specificity and agreement of AFB microscopy. Sensitivity was 98.0%, specificity was 99.7% and agreement was 99.2%. Furthermore, to predict the sensitivity of the laboratory, and based on the results obtained in the present study, the following formula was generated using least squares regression techniques:

$$\% \text{ sensitivity} = 79.6 + (3.54 \times \text{number of low positives correctly identified}) + (1.77 \times \text{number of strong positives and negatives correctly identified}).$$

This formula demonstrates the importance of the low-positive PT slides being correctly identified in predicting the sensitivity of the laboratory, as each is worth twice the value of any other slide in predicting the estimated sensitivity of the laboratory.

Table 4 shows comparative PT results for 1998 and 2001 by false-negative and false-positive results, quantification errors and concordant slides of 430 microscopists who performed both tests: a) shows overall data, b) shows data by laboratory type and c) by qualification. Table 5 pools the statistical analysis of all data shown in Table 4. Significant differences were obtained in all analysed comparisons of 1998 vs. 2001, and none were found in comparisons performed in 1998 or 2001, except errors associated with type of degree (chemists vs. technicians), which showed significant differences only in 1998.

Table 5 Statistical analyses of Table 4

Parameter	χ^2	*P* value
Any error, 1998 vs. 2001	49.78	0.001
Agreement, 1998 vs. 2001	53.34	0.001
SPHL, 1998 vs. 2001	19.26	0.001
Hospital, 1998 vs. 2001	13.55	0.008
Health centre, 1998 vs. 2001	32.44	0.001
Chemist, 1998 vs. 2001	46.21	0.001
Technician, 1998 vs. 2001	8.91	0.06
Laboratory type, any error		
1998	3.18	0.92
2001	16.2	0.04
Laboratory type, agreement		
1998	1.41	0.49
2001	3.68	0.055
Chemist vs. technician		
Any error, 1998	12.75	0.01
Agreement, 1998	2.12	0.14
Any error, 2001	0.64	0.95
Agreement, 2001	0.58	0.44

SPHL = State Public Health Laboratory.

DISCUSSION

This nationwide study included all microscopists who perform AFB diagnosis in the public health system as well as all the rechecking information obtained in 1998, as the study was intended to compare the established EQA system, i.e., rechecking, with proficiency

testing. PT implied acquiring the expertise to prepare slides with known numbers of bacilli and producing hundreds of slide sets. We therefore had some handicaps, for example the slide sets produced in 1998 had random numbers of negative, low-positive and 1+ and 2+ slides. An analysis of the results showed that the number of low-positive slides in the set was the factor most closely associated with PT results. Subsequent sets therefore had defined numbers of different graded slides. Non-blinded and possible sampling bias in rechecking was also a limitation found in the system, which was suggested by the very high agreement in rechecking and the very low number of laboratories with errors found; this reinforces the importance of PT. Finally, we could not be certain if the time used for PT was the same when the sets were sent by courier as when they were performed in the presence of a supervisor.

In spite of the limitations mentioned, this study showed that training technicians proved an essential component in successfully implementing proficiency testing in the national network of TB laboratories, as demonstrated by the significant improvement in PT scores after microscopists completed a 3-day training course, and confirmed by the comparison of PT from 1998 and 2001. Attending a training course at a national laboratory might also be motivating.[8–10] It was also interesting that even when PT was sent by courier, improvements were detected as a result of the training course conducted at InDRE: in 1998 no significant differences were found in PT scores associated with laboratory type and professional degree, probably due to a general lack of training. In 2001, PT scores were significantly higher in SPHL than other types of laboratories, probably because there are more academic activities at SPHL and the personnel read more slides, supervise local laboratories through rechecking and have better facilities.[8,10] On the other hand, chemists had significantly lower scores than laboratory technicians, possibly due to the fact that they are in charge of AFB culture while technicians are dedicated to AFB microscopy and are more motivated. Furthermore, in the past, local laboratories had minimal access to information, guidelines and sometimes training. These alternatives need to be evaluated in future studies.

Various laboratory quality factors were found to be associated with PT. Due to the seemingly incongruency of these factors, an analysis for confounders was performed; 'easy' slides were associated with a better score, in agreement with the finding that when more low positive slides, i.e., 'difficult' slides, were included in the test, lower PT sores were obtained. The total number of slides was also significant and therefore measured the impact of our decision to use PT results only when more than five slides were read. Finally, in 2001 laboratory type was also significant, in accordance with the results of significant differences among PT scores associated with laboratory type in 2001.

Of the 637 laboratories in the National Network, only 109 (17%) had complete rechecking data and reported any errors, indicating the difficulty of properly implementing EQA based on rechecking. Added to the labour-intensiveness of rechecking, there could also be a bias in the selection of slides sent for rechecking. Furthermore, microscopists in the SPHL could have less incentive to recheck if the slides are received already labelled as negative or positive.[10] Laboratories also may not submit slides for rechecking because they have concerns about the implications of their low performance. Small local laboratories may also lack a relationship with the SPHL, implying that state laboratories may need to improve their networks. In spite of all of these potential problems, technicians working in laboratories using rechecking had significantly higher PT scores. One can conclude that being in an EQA programme improves performance. The difficulty in finding an association between PT and rechecking was partially due to the fact that the PT slide sets used did not all have the same level of difficulty, as measured by the number of low positives in each set. In addition, only a small percentage of the laboratories had complete rechecking of data with any reported errors. One can surmise that laboratories that had poor PT performance simply did not submit slides for rechecking or had incomplete or unreliable data that could not be used in the analysis.

The comparison of PT and rechecking with laboratory factors may indicate that general laboratory equipment, maintenance and supplies are associated with performance. However, the relationship is unclear, as some of the factors found to be significant are not directly related to AFB microscopy, while others found to be non-significant are related to AFB microscopy.

The results of this study are in agreement with other studies where the factor most strongly associated with PT performance was the number of low-positive slides in the slide sets.[9,10] The obvious conclusion is that all PT slide sets should have the same number of low-positives, strong-positives and negatives to consistently measure performance. Error rates were higher in slides with low numbers of AFB, and therefore culture of such samples has been reinforced in Mexico. These results might suggest that microscopists are unable to detect low AFB counts, probably because they do not read all fields, or due to the high turnover rate, and therefore the outcome is a false-negative result. This kind of error is important, as patients with paucibacillary disease could give negative results in AFB microscopy and will therefore not receive treatment, resulting in further community spread and failure in diagnosis of pulmonary TB.[10]

In conclusion, our study indicates that it is possible

for a national reference laboratory to prepare consistent PT slide sets for hundreds of microscopists. PT also allowed the effect of training on the performance of microscopists to be evaluated. Lastly, there was an association between PT and rechecking, suggesting that PT or rechecking can be used for EQA, and that using both methods may be beneficial.

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RÉSUMÉ

CONTEXTE : Le diagnostic de tuberculose (TB) dans les pays en développement repose sur l'identification de bacilles acido-résistants (BAAR) dans les frottis.
OBJECTIF : Afin d'évaluer la qualité de l'examen microscopique à la recherche des BAAR, le laboratoire national de référence de Mexico a mis en œuvre des tests de compétence (TC) dans un réseau de 637 laboratoires du Secrétariat de la Santé.
SCHÉMA : On a supervisé au total 586 laboratoires (92%) et évalué 430 techniciens dans un TC consistant en l'examen de 10 lames dont chacune comportait des nombres connus de BAAR. Les résultats ont été comparés à ceux du réexamen des lames et à ceux des TC menés 2 ans plus tard.
RÉSULTATS : Des 430 techniciens évalués par le TC en 1998, les scores étaient inférieurs à 80% chez 196 (46%), qui ont subi une formation intensive en 1999. D'une moyenne de 65%, les résultats ont augmenté à 90% ($P < 0{,}0001$). En 2001 ils ont été réévalués par le TC, avec un score moyen de 83%. Les facteurs principaux affectant le score du TC furent le type de laboratoire où travaillaient les microscopistes ainsi que le nombre de lames faiblement positives (1–9/100) dans le test. Les laboratoires dont le travail était recontrôlé avaient de meilleurs scores de TC ($P = 0{,}002$). On a trouvé une association entre le score du TC et la sensibilité estimée du laboratoire du microscopiste ($P = 0{,}01$).
CONCLUSION : L'évaluation externe de qualité et la formation améliorent la performance du diagnostic, tandis que le réexamen des lames et les tests de compétence sont deux mesures applicables de la performance des laboratoires.

RESUMEN

MARCO DE REFERENCIA : En los países en desarrollo, el diagnóstico de la tuberculosis (TB) se basa en la identificación de bacilos alcohol-ácido resistentes (BAAR) en los frotis de expectoración (baciloscopias).
OBJETIVO : Con el fin de evaluar la calidad del examen microscópico para BAAR, el laboratorio nacional de referencia de México implementó pruebas de competencia (PC) en una red de 637 laboratorios de la Secretaría de Salud.
MÉTODO : Se supervisó un total de 586 (92%) laboratorios y 430 técnicos en una PC que consistía en la lectura de 10 frotis, cada uno con un número conocido de BAAR. Los resultados fueron comparados con aquéllos del reexamen de los frotis y de una PC implementada 2 años más tarde.
RESULTADOS : De los 430 técnicos evaluados por la PC, los calificaciones fueron inferiores a 80% en 196 (49%), quienes recibieron un entrenamiento intensivo en 1999. De una calificación promedio de 65%, hubo un aumento hasta 90%. En 2001 fueron evaluados otra vez por la PC, con una calificación promedio de 83%. Los principales factores que afectaron la calificación de la PC fueron el tipo de laboratorio donde trabajaba el microscopista y el número de frotis con bajo grado de positividad (1–9/100) en la prueba. Las mejores calificaciones de la PC se encontraron en los laboratorios donde el trabajo era revisado ($P = 0{,}002$). Se encontró una asociación entre la calificación de la PC y la sensibilidad estimada del laboratorio del microscopista ($P = 0{,}01$).
CONCLUSIÓN : El aseguramiento externo de calidad y el entrenamiento mejoran el rendimiento diagnóstico. El reexamen y la prueba de competencia son dos medidas aplicables para mejorar el rendimiento del laboratorio.