INT J TUBERC LUNG DIS 10(9):1030–1035

Efficiency of serial smear examinations in excluding sputum smear-positive tuberculosis

B. Mabaera,* N. Naranbat,† P. Dhliwayo,‡ H. L. Rieder‡

*** University of Zimbabwe, Harare, Zimbabwe; † National Center for Communicable Diseases, Ministry of Health, Ulaanbaatar, Mongolia; ‡ International Union Against Tuberculosis and Lung Disease, Paris, France**

SUMMARY

SETTING: This study determined the number of slides required to identify one additional case of sputum smear-positive tuberculosis (TB) from the third smear. The study hypothesis was that not more than 100 and 75 slides, respectively, in Mongolia and Zimbabwe, need to be examined to find one additional case of TB with a third serial diagnostic sputum smear examination.

METHODS: In a retrospective, record-based study, data were abstracted from TB laboratory registers from all 31 laboratories in Mongolia and 23 randomly selected laboratories in Zimbabwe using a uniform EpiData collection instrument.

RESULTS: A total of 52 909 records of examinees were available. In Mongolia, of 15 103 suspects, 1717 (11.4%) were positive. Of these, 0.7% were positive for the first time on the third smear examination. In Zimbabwe, of 25 693 suspects, 3452 (13.4%) were positive and 4.5% were positive only on the third smear examination. The expected number of slides required to detect one additional case on the third examination was 1153.3 for Mongolia and 132.6 for Zimbabwe.

CONCLUSIONS: The requirement of routine examination of three serial smears before declaring a suspect as a 'non-case' (of sputum smear-positive TB) will need to be reviewed in both Mongolia and Zimbabwe.

KEY WORDS: tuberculosis; diagnosis; laboratory; microscopy

IN VARIOUS STUDIES in different settings, it has been observed that the majority of persons with suspected tuberculosis (TB) ultimately found to have sputum smear-positive TB were positive already on first smear examination, and that there is a rapidly diminishing return with serial smears.[1–5] The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (The Union) recommend the examination of three smears before declaring a person with suspected TB smear-negative.[6–8] It is not clear whether this recommendation is applicable and appropriate for every setting. Although some diagnostic benefit might be gained from performing three serial smears, the added workload can be counterproductive and result in a lower quality service that may affect the efficiency of the critical first two sputum smear examinations. It is ultimately a cost-effectiveness decision to determine the maximum number of slides required to identify one additional case of sputum smear-positive tuberculosis.

This issue was discussed at national level in Mongolia and Zimbabwe, and a decision was reached and endorsed on a critical number that should not be exceeded. The critical numbers of 100 and 75 were agreed for Mongolia and Zimbabwe, respectively. The resulting hypothesis was then tested on nationally representative samples of laboratory registers.

METHODS AND MATERIALS

Hypothesis

The study hypothesis was stated as follows:

> $H_{0\text{-}d}$: not more than 100 and 75 slides in Mongolia and Zimbabwe, respectively, need to be examined to find one additional case of TB with a third serial diagnostic sputum smear examination.

In case of refutation, the requirement of routine examination of three serial smears before declaring a suspect as a 'non-case' (of sputum smear-positive TB) will be discontinued.

Definition of a case

For the purpose of this study, once any acid-fast bacilli (AFB) were identified on any smear examination, the examinee was defined as a 'case' of smear-positive TB. In those instances, subsequent results were not taken into consideration.

Correspondence to: Biggie Mabaera, P O Box CY1722, Causeway, Harare, Zimbabwe. Tel: (+263) 9-738 048. Fax: (+263) 9-738 048. e-mail: bmabaera@comone.co.zw

Article submitted 20 January 2006. Final version accepted 22 May 2006.

[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.iuatld.org]

Design and data source

This was a retrospective, record-based study, utilising information routinely available in national TB control services. Records that were not currently in use for the period January–December 2002 or the most recent previous calendar year were utilised.

Sampling

In both countries, laboratories that offered TB diagnostic services using sputum microscopy and had a standard TB laboratory register were selected. All diagnostic laboratories were enumerated in each country, and given an identification code. From this list, 30 of 95 laboratories in Zimbabwe were randomly selected to ensure an unbiased and representative evaluation of the country's laboratories. All 31 laboratories in Mongolia were selected.

Data collection

A record was made of the number of patients registered in the TB register of the selected laboratories. Data collection was limited to the following variables: TB laboratory code, laboratory serial number, registration date, sex, age, reason for examination and the results of each of the three possible examinations. Information on 10 variables was thus recorded for each examinee. A unique identifier for each examinee was automatically created from the TB laboratory code, the laboratory serial number and the year of registration in which the examination was done. This unique identifier was essential for validating records following double entry.

Data entry, validation and analysis

A uniform questionnaire was used in the data collection process, and data from each laboratory were entered twice using EpiData (version 3.1, EpiData Association, Odense, Denmark, 2005). The two files from each laboratory were validated by comparison of the files using the unique identifier in both data sets. The discordant records were examined in the original hard copy laboratory register, and a final corrected data set was produced. The different files were subsequently appended for analysis.

All analyses were performed using EpiData Analysis and a spreadsheet (OpenOffice 1.1., Sun Microsystems, Beaverton, OR, 2004). The primary objective was to determine the number of slides that need to be examined to identify one additional case of sputum smear-positive TB on the third diagnostic examination. Elements used to provide the basis for testing the hypothesis include the calculation of the proportion of cases among suspects and the incremental yield from serial diagnostic smears. The method used in a previous study by Ipuge et al. in Tanzania[3] and by Rieder et al.[9] was used to compute the incremental yield from the first, second and third smear examinations. To calculate credibility intervals around the number of smears required to identify one additional case with diagnostic sputum smear examinations, a Bayesian analysis was performed using the Markov Chain Monte Carlo approach (WinBUGS, Imperial College & Medical Research Council, London, UK, Version 1.4, 2003). The reasoning for this approach is that while the random selection process was representative, there may be considerable uncertainty around such small proportions, despite the large sample size. Thus, the basis of analysis was the country, and not the individual laboratories.

RESULTS

Characteristics of examinees

All the 31 laboratories in Mongolia and 23 in Zimbabwe were included in the study. While 30 of the 95 laboratories in Zimbabwe were randomly selected, seven were inaccessible, leaving 23. A total of 57 343 records were available. Of these, 307 (0.5%, 43 in Mongolia and 264 in Zimbabwe) were excluded due to an erroneous laboratory code, a first result recorded as unknown or a second recorded as unknown but followed by a valid result. Of the remaining 57 036 records, 4127 (7.2%, 58 in Mongolia and 4069 in Zimbabwe) were excluded because the laboratory register did not state the reason for the sputum examination. Thus, a total of 52 909 records were retained for analysis.

The distribution of examinees by country, reason for examination, sex and age group is summarized in Table 1. In Mongolia, the proportion of examinees with a diagnostic examination among all examinees was considerably smaller (67.1%) than in Zimbabwe (84.5%).

The age distribution was remarkably similar in both countries. The mean and median ages were 35.9 and 33.0 years, respectively, in Mongolia compared to 35.1 and 33.0 years in Zimbabwe. In Mongolia, there were fewer female (47.0%) than male examinees, while in Zimbabwe there was a female preponderance (50.5%).

Characteristics of suspects

In Mongolia, of the 15 103 suspects, 1717 (11.4%) were cases. In Zimbabwe, of the 25 693 suspects, 3452 (13.4%) were cases. The distribution of cases by country, sex and age group is shown in Table 2. The proportion of cases among suspects by sex was similar in both countries. As expected, the yield of cases was lowest among children among the age groups. The largest proportion of cases was found in those aged 15–34 years.

Incremental yield from serial smears

Of the 13 386 suspects who were negative on smear examination in Mongolia, 3.1% were negative on one smear examination, 5.3% on two smear examinations, and 91.6% on three smear examinations (Table

Table 1 Proportion of suspects among examinees with known reason for examination, Mongolia and Zimbabwe, 2002

	Mongolia				Zimbabwe			
	Diagnosis				Diagnosis			
Characteristic	Number	Fraction	Follow-up	Total	Number	Fraction	Follow-up	Total
Total	15 103	0.671	7 394	22 497	25 693	0.845	4 719	30 412
Age, years								
0–14	986	0.793	257	1 243	1 022	0.891	125	1 147
15–24	3 165	0.619	1 949	5 114	2 259	0.815	512	2 771
25–34	3 355	0.620	2 053	5 408	7 301	0.814	1 668	8 969
35–44	2 833	0.651	1 517	4 350	5 220	0.830	1 069	6 289
45–54	1 760	0.709	724	2 484	2 360	0.844	437	2 797
55–64	1 278	0.762	400	1 678	1 059	0.857	176	1 235
⩾65	1 467	0.844	271	1 738	667	0.883	88	755
Unknown	259	0.537	223	482	5 805	0.900	644	6 449
Sex								
Female	7 120	0.681	3 337	10 457	12 853	0.857	2 136	14 989
Male	7 875	0.663	4 010	1 185	12 100	0.839	2 328	14 428
Unknown	108	0.697	47	155	740	0.744	255	995

3). Of the 22 241 suspects who were negative on smear examination in Zimbabwe, 8.1% were negative on one smear examination, 12.2% on two smear examinations, and 79.8% on three smear examinations.

Of 1717 suspects who were positive on smear examination in Mongolia, 96.9% were positive for the first time on the first smear examination, 2.4% on the second smear examination, and 0.7% on the third smear examination. Of 3452 suspects who were positive on smear examination in Zimbabwe, 86.1% were positive for the first time on the first smear examination, 9.4% on the second smear examination, and 4.5% on the third smear examination.

Cases missed due to incomplete examination series among negative suspects were estimated from the complete series with at least one positive result. Adding the missed to the observed cases provided the appropriate denominator to re-calculate the expected yield if all examinations had in fact been done. Thus, the potential yield from the first examination was 96.7% and 84.5% in Mongolia and Zimbabwe, respectively, the second added 2.5% and 10.0%, and the third added 0.8% and 5.5% cases that had been missed by earlier examinations.

Study hypothesis

Using the prevalence of cases among suspects multiplied by the incremental yield on the third smear provides the overall fraction positive on the third smear only, and its reciprocal value, the number of smears required to find a case on the third serial smear examination. The expected number of slides required to detect one additional case on the third examination was therefore 1153.3 for Mongolia and 132.6 for Zimbabwe. Because of the small fractions calculated,

Table 2 Number and proportion of cases among suspects, Mongolia and Zimbabwe, 2002

	Mongolia		Zimbabwe	
Characteristic	Cases	Fraction*	Cases	Fraction*
Total	1717	0.114	3452	0.134
Age, years				
0–14	28	0.028	74	0.072
25–34	511	0.152	1160	0.159
35–44	372	0.131	714	0.137
45–54	161	0.091	310	0.131
55–64	102	0.080	97	0.092
⩾65	60	0.041	39	0.058
Unknown	483	0.141	1058	0.131
Sex				
Female	823	0.116	1610	0.125
Male	889	0.113	1743	0.144
Unknown	5	0.046	99	0.134

* The denominators are shown in the columns 'Diagnosis' in Table 1.

Table 3 Pattern of serial sputum smear examinations among suspects, missed cases, corrected proportion of cases among suspects and incremental yield from serial smears, Mongolia and Zimbabwe, 2002

	Mongolia	Zimbabwe
Observed pattern*		
Total	15 103	25 693
NNN	12 264	17 740
*NN*9	708	2 706
*N*99	414	1 795
Px	1 663	2 972
NPx	42	325
NNP	12	155
Missed cases		
Second examination	1.3	27.9
Third examination	1.1	38.7
Expected yield (if 3 smears examined)		
Observed + missed cases	1.000	1.000
Expected from first smear	0.967	0.845
Expected from second smear	0.025	0.100
Expected from third smear	0.008	0.055

* *NNN* = all three smears examined and negative; *NN*9 = first two smears examined and negative, third not examined; *N*99 = first smear examined and negative, second and third not examined; *Px* = first smear positive; *NPx* = first smear negative, second positive; *NNP* = first and second smear negative, third positive.

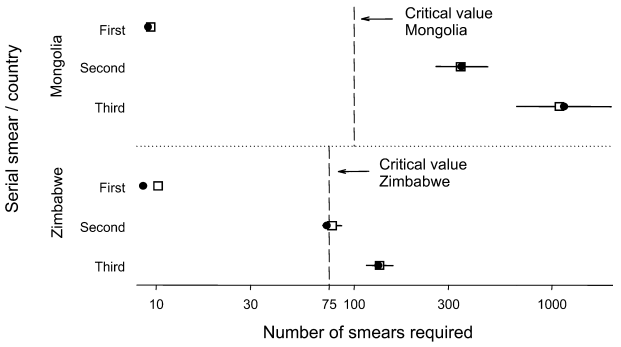

Figure Point estimates and 95% credibility interval for the number of suspects needing to be examined to identify one additional case of TB with serial smears, Mongolia and Zimbabwe, 2002. Filled circles are means, hollow squares medians with 95% credibility intervals shown as solid horizontal lines. Vertical dashed lines indicate critical values for Mongolia (100 smear examinations) and Zimbabwe (75 smear examinations) to identify one additional case on a third serial smear examination. TB = tuberculosis.

a Bayesian approach was chosen to estimate the uncertainty around the point estimates. The modelled credibility interval for the number of smears to be examined to identify one additional case was 659–1996 (with a mean and median of 1147 and 1086, respectively) for Mongolia. For Zimbabwe, the mean and median were respectively 135 and 135, with a credibility interval of 116–158 (Figure).

With a maximum number of 100 slides as the critical value for Mongolia, the study hypothesis is thus refuted. In Zimbabwe, with a critical value of 75 slides, the study hypothesis is also refuted.

DISCUSSION

Sputum microscopy is a cornerstone in the diagnosis of pulmonary tuberculosis (PTB). All individuals who present to the general health services who are likely to have TB are required to undergo a sputum smear examination. Information on patients whose sputum is examined is entered in the Tuberculosis Laboratory Register.[6] Although the TB laboratory registers in the field that are used in routine microscopy work suffer to a various degree from a multitude of problems, they nevertheless provide all the information upon which a national tuberculosis programme (NTP) must rely. In this study, we looked at the efficiency of examining serial sputum smears under routine conditions. The choice of applying exactly the same protocol in two entirely different settings was arbitrary, yet the results were remarkably similar. Both countries had a policy of 'spot-morning-spot' collection at the time of the study, but it is unclear to what extent this policy was being implemented.

In several studies it has been observed that with three serial smears, about 85% of ultimately positive suspects are positive on the first, an additional 10% on the second, and around 5% on the last sputum smear examination. This observation has nevertheless not been consistent, and deviations have not been uncommon. In this study, 99% of cases in Mongolia and 96% in Zimbabwe were positive on the first two slides. The high yield of the first two smears is consistent with findings from other studies.[3,10–14] Conversely, the yield and incremental benefit of the third smear was very small in Zimbabwe and even smaller in Mongolia. The very small yield from the third smear has two major possible explanations: it is possible that the technicians were diligently examining each smear and thus identified virtually all cases with two smears. Alternatively, as has been seen in other settings,[9] the contrary could be the case: overburdened technicians may be much less diligent on examining a third smear following two negative smears.

A study by Harries et al. compared a strategy for screening TB suspects with two sputum smears and three sputum smears for different 6-month periods.[15] In the two 6-month periods, the same proportion (16%) of suspects was sputum smear-positive. In Zambia, among TB suspects who had TB, 92% were positive on the first two smear examinations.[16] Blair et al. looked at the efficacy of sputum smear examination under ideal conditions where each examinee had at least seven serial sputum smear examinations, each adding some, but successively less, yield.[1] Examining more sputum samples may be counterproductive in the sense that the overburdened technician may examine too few fields or copy the result of the first smear examination into the result columns for the second and third sputum smears.[17]

Concerns might be raised that a small proportion of cases may be missed by not examining the third serial smear. However, sputum smear-negative, symptomatic TB suspects are investigated further by radiography, trials of antimicrobial therapy for alternative diagnoses, and additional sputum smear examination if there is no clinical improvement. By examining two sputum specimens, the sensitivity of sputum smear examination might actually improve as the technician will have fewer slides to prepare and examine. Whether this will imply two morning specimens or one spot specimen plus one morning specimen will need to be discussed and decided by the authorities.

CONCLUSIONS

The effectiveness of the third serial smear was low in both countries. The requirement for routine examination of three serial smears before declaring a suspect as a 'non-case' (of sputum smear-positive TB) will need to be reviewed in Mongolia and Zimbabwe. A routine external quality assessment programme has been demonstrated to improve the quality of sputum smear microscopy.[18] It therefore seems imperative that the small loss in detection of smear-positive cases

due to a reduction in the number of serial examinations routinely required is compensated by increasing the efficacy of the remaining examinations. In practical terms, this means rapid implementation of external quality assessment programmes in both countries.

Acknowledgements

This paper is an outcome of a Union operations research course conducted in January 2004. The course was supported by the United States Agency for International Development under the terms of Award No. HRN-a-00-00-00018-00.

The ten participants in the course were Dr M Nasehi (Iran), Dr N Naranbat (Mongolia), Dr M Occhi (Mozambique), Dr G Balasanyants (Russia), Dr Y Shubin (Russia), Pr N Beyers (South Africa), Ms K Lawrence (South Africa), Ms A Wright (World Health Organization, Switzerland), Ms W Hassan Ali Taha (Sudan), and Dr B Mabaera (Zimbabwe). The course was facilitated by Dr P Dhliwayo (The Union), Dr J M Lauritsen (EpiData Association), Dr R Makombe (World Health Organization, Zimbabwe), and Dr H L Rieder (The Union). Most of the participants and all the facilitators provided input during the drafting of the research protocol.

The course project relied entirely on the collection of data by the use of EpiData Entry and the analysis by the use of EpiData Analysis. Throughout this post-course project, the authors benefited from the input and counsel provided by Dr J M Lauritsen.

Dr B E Neuenschwander kindly provided the WinBUGS script to determine credibility intervals.

B Buyankhishig, Head of Reference TB Laboratory, National Center for Communicable Diseases, Mongolia (NCCD), Dr B Tserelmaa, S Darisuren, N Amarjargal, NCCD, provided technical and financial support for the study in Mongolia.

Dr D Dhlakama, Dr S Midzi, Dr O Mugurungi, Dr T Apollo and Mr N Siziba in the Ministry of Health & Child Welfare, Zimbabwe, provided technical and financial support towards the project.

References

1 Blair E B, Brown G L, Tull A H. Computer files and analyses of laboratory data from tuberculosis patients. II. Analyses of six years' data on sputum specimens. Am Rev Respir Dis 1976; 113: 427–431.
2 Urbanczik R. On the possibility of cross-resistance between rifampicin and clofazimine in mycobacteria—a laboratory study. Zbl Bakt Hyg A 1985; 260: 113–116.
3 Ipuge Y A I, Rieder H L, Enarson D A. The yield of acid-fast bacilli from serial smears in routine microscopy laboratories in rural Tanzania. Trans R Soc Trop Med Hyg 1996; 90: 258–261.
4 Rieder H L, Arnadottir T, Tardencilla Gutierrez A A, et al. Evaluation of a standardized recording tool for sputum smear microscopy for acid-fast bacilli under routine conditions in low income countries. Int J Tuberc Lung Dis 1997; 1: 339–345.
5 Nelson S M, Deike M A, Cartwright C P. Value of examining multiple sputum specimens in the diagnosis of pulmonary tuberculosis. J Clin Microbiol 1998; 36: 467–469.
6 Enarson D A, Rieder H L, Arnadottir T, Trébucq A. Management of tuberculosis. A guide for low income countries. 5th ed. Paris: International Union Against Tuberculosis and Lung Disease, 2000.
7 World Health Organization. Laboratory services in tuberculosis control. Part II: microscopy. Geneva, Switzerland: WHO, 1998.
8 World Health Organization. Tuberculosis handbook. WHO/TB/98.253. Geneva, Switzerland: WHO, 1998.
9 Rieder H L, Chiang C Y, Rusen I D. A method to determine the utility of the third diagnostic and the second follow-up sputum smear examination to diagnose tuberculosis cases and failures. Int J Tuberc Lung Dis 2005; 9: 384–391.
10 Crampin A C, Floyd S, Mwaungulu F, et al. Comparison of two versus three smears in identifying culture-positive tuberculosis patients in a rural African setting with high HIV prevalence. Int J Tuberc Lung Dis 2001; 5: 994–999.
11 Wu Z L, Wang A Q. Diagnostic yield of repeated smear microscopy examinations among patients suspected of pulmonary TB in Shandong province of China. Int J Tuberc Lung Dis 2000; 4: 1086–1087.
12 Cascina A, Fietta A, Casali L. Is a large number of sputum specimens necessary for the bacteriological diagnosis of tuberculosis? [Correspondence]. J Clin Microbiol 2000; 38: 466.
13 Mathew P, Kuo Y H, Vazirani B, Eng R H K, Weinstein M P. Are three sputum acid-fast bacillus smears necessary for discontinuing tuberculosis isolation? J Clin Microbiol 2002; 40: 3482–3484.
14 Sarin R, Mukerjec S, Singla N, Sharma P P. Diagnosis of tuberculosis under RNTCP: examination of two or three sputum specimens. Indian J Tuberc 2001; 48: 13–16.
15 Harries A D, Mphasa N B, Mundy C, Banerjee A, Kwanjana J H, Salaniponi F M L. Screening tuberculosis suspects using two sputum smears. Int J Tuberc Lung Dis 2000; 4: 36–40.
16 Walker D, McNerney R, Kimankinda Mwembo M, Foster S, Tihon V, Godfrey-Faussett P. An incremental cost-effectiveness analysis of the first, second and third sputum examination in the diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis 2000; 4: 246–251.
17 Van Deun A, Hamid Salim A, Cooreman E, et al. Optimal tuberculosis case detection by direct sputum smear microscopy: how much better is more? Int J Tuberc Lung Dis 2002; 6: 222–230.
18 Aziz M A, Ba F, Becx-Bleumink M, et al. External quality assessment for AFB smear microscopy. Ridderhof J, Humes R, Boulahbal F, eds. Washington, DC: Association of Public Health Laboratories, 2002.

RÉSUMÉ

CONTEXTE : Cette étude a déterminé le nombre de lames nécessaires pour identifier un cas supplémentaire de tuberculose (TB) à bacilloscopie positive des expectorations à partir du troisième frottis. L'hypothèse d'étude était la suivante : il ne faut pas plus de 100 ou de 75 lames respectivement en Mongolie et au Zimbabwe pour que l'examen décèle un cas supplémentaire de TB au moyen de l'examen en série d'une troisième expectoration pour diagnostic.

MÉTHODES : Il s'agit d'une étude rétrospective basée sur les dossiers. Les données proviennent des registres de laboratoire de TB de l'ensemble des 31 laboratoires de Mongolie et de 23 laboratoires sélectionnés au hasard au Zimbabwe ; on a utilisé un instrument uniforme EpiData pour les rassembler.

RÉSULTATS : Au total, 52 909 dossiers de sujets examinés étaient disponibles. En Mongolie, sur 15 103 suspects, 1717 (11,4%) étaient positifs. Parmi ces derniers, 0,7% ont été positifs pour la première fois lors du troisième examen de frottis. Au Zimbabwe, sur 25 693 suspects, 3452 (13,4%) étaient positifs et 4,5% ne l'ont été que lors du troisième examen de frottis. Le nombre

attendu de lames nécessaires pour détecter un cas supplémentaire lors du troisième examen a été de 1153,3 en Mongolie et de 132,6 au Zimbabwe.
CONCLUSIONS : L'exigence d'un examen de routine de trois frottis en série avant de déclarer un suspect comme un 'non-cas' (de TB à bacilloscopie positive) devra être révisée tant en Mongolie qu'au Zimbabwe.

RESUMEN

MARCO DE REFERENCIA : El presente estudio buscó determinar el número de frotis necesarios, para diagnosticar un nuevo caso de tuberculosis (TB) con baciloscopia positiva. Según la hipótesis de trabajo, este número crítico de frotis no debía ser superior a 100 en Mongolia y a 75 en Zimbabwe, a fin de diagnosticar un caso más de TB mediante baciloscopias seriadas con tres muestras.
MÉTODOS : Fue este un estudio retrospectivo con base en las historias clínicas. Los datos se obtuvieron a partir de los registros de los 31 laboratorios de TB de Mongolia y de 23 laboratorios escogidos aleatoriamente en Zimbabwe, mediante el instrumento de recolección uniforme de datos EpiData.
RESULTADOS : Se contó con un total de 52 909 historias de pacientes examinados. En Mongolia, de 15 103 personas con presunta TB, 1717 (11,4%) tuvieron baciloscopia positiva. De estas el 0,7% tuvo una primera baciloscopia positiva en el tercer frotis examinado. En Zimbabwe hubo 25 693 personas examinadas, de las cuales 3452 (13,4%) tuvieron resultados positivos y 4,5% presentaron una baciloscopia positiva sólo en el tercer frotis examinado. El número previsto de frotis requeridos a fin de detectar un caso adicional en la tercera muestra fue de 1153,3 en Mongolia y de 132,6 en Zimbabwe.
CONCLUSIONES : Es necesario revisar el requisito del examen sistemático de tres muestras seriadas de esputo antes de declarar una persona con presunta TB un 'non caso' (de TB con baciloscopia positiva) en Mongolia y en Zimbabwe.