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The yield of acid-fast bacilli from serial smears in routine microscopy laboratories in rural Tanzania

Yahya A. I. Ipuge[1], Hans L. Rieder[2] and Donald A. Enarson[2] *[1]National Tuberculosis/Leprosy Programme, Ministry of Health, Dar es Salaam, Tanzania; [2]International Union Against Tuberculosis and Lung Disease, Paris, France*

Abstract

Routine results of direct examination of sputum smears for acid-fast bacilli from 34 laboratories in Tanzania were analysed. These represented 8 regions providing 94 laboratory-years of work; 61 580 tuberculosis suspects were evaluated with the aid of 141 371 smears. The average proportion of cases found among suspects was 18·9% (range 14·3–23·8% in the 8 regions). The number of cases missed among suspects with incomplete examinations was calculated based on the number observed among suspects with a complete set of 3 smears examined, and an incremental yield of 83·4% with the first, 12·2% with the second, and 4·4% with the third smear was estimated for the total number of expected cases. These data suggest that (i) the method frequently employed for calculating requirements for laboratory supplies in low income countries, based on the estimate that 10 suspects need examination to identify one case of sputum smear-positive tuberculosis, is generous in the context of Tanzania and (ii) under routine conditions the incremental yield from a third smear examination after 2 negative examinations is relatively small.

Keywords: tuberculosis, diagnosis, serial smears

Introduction

Sputum smear examination by microscopy for acid-fast bacilli remains the cornerstone of tuberculosis case-finding in low-income countries. It is well known (SHAW & WYNN-WILLIAMS, 1954; GRYZBOWSKI *et al.*, 1975; VAN GEUNS *et al.*, 1975) that the most infectious cases of tuberculosis are those with sufficient concentration of *Mycobacterium tuberculosis* to be visible by direct microscopical examination of sputum smears. These examinations are relatively easy to perform with a high degree of reliability (TOMAN, 1979). Moreover, because most patients with infectious tuberculosis have symptoms, the use of smear microscopy in those presenting to health services with suggestive symptoms is the most efficient means of case detection (STYBLO *et al.*, 1967). The efficiency of smear examination in detecting cases of tuberculosis subsequently shown to be culture positive is known (CHAN *et al.*, 1971), and the high specificity of a positive sputum smear predicting *M. tuberculosis* has been confirmed even under circumstances where disease due to environmental mycobacteria is very prevalent (DEBRUNNER *et al.*, 1992; YAIKO *et al.*, 1994). In the present study, the number of smears examined per case detected and the yield from serial smear examinations under routine programme conditions were evaluated in rural Tanzania.

Materials and Methods

The National Tuberculosis and Leprosy Programme in Tanzania uses specifically designed laboratory registers for acid-fast smear microscopy recommended by the International Union Against Tuberculosis and Lung Disease (IUATLD) (ENARSON *et al.*, 1994) to record the results of every patient undergoing examination in the district. Each patient examined for tuberculosis is assigned a single line in the register. Two columns indicate whether the subject is a new suspect or a known tuberculosis patient on treatment with an examination for follow-up of treatment, information that is obtained from the request form for sputum smear examination. A new suspect is defined as a patient currently not receiving antituberculosis treatment with a persistent cough of 3 or more weeks' duration or a patient presenting with other persistent clinical symptoms commonly associated with tuberculosis, such as chest pain, fever, shortness of breath, or loss of weight (TANZANIA, 1987). In Tanzania, virtually all microscopical examinations for acid-fast bacilli are made on sputum specimens and the fraction of specimens examined from extra-respiratory sites is so minute as to be negligible. The recommended practice is examination of 3 sputum specimens by the Ziehl–Neelsen method. The results are entered in 3 separate columns on the same line.

Eight of the 19 regional co-ordinators outside the large city of Dar es Salaam agreed to participate. Each of them was requested to collect information from at least 5000 consecutive suspects and, once this target was achieved, to complete the year under evaluation. More than one laboratory was included in each region. The 27 possible combinations of results from 3 examinations (negative, positive, not done) are reduced to 14 observable patterns only, because the result 'not done' can only succeed, but not precede, a 'negative' or 'positive' result. Each of these 14 possible patterns of laboratory results for all new tuberculosis suspects for each given year and laboratory was abstracted.

The tabulated data were entered in a computer for analysis with commercially available software (FoxPro® relational database management system for MS-DOS, version 2.5, Microsoft Corporation, 1993).

Each single year within each laboratory was treated as a separate record. From this data set the total number of smears performed per region, the total number of smears performed per identified smear-positive case (within this study defined as a suspect with at least one positive smear examination), the average number of smears performed per working day (assuming a 5 d working week throughout the year), and the number of suspects examined to identify a single smear-positive case, were calculated.

The data set was then collapsed to provide the 6 essential patterns (see Appendix) for analysis of the number of cases identified from the first smear among all suspects, the number of cases first identified from the second smear among all who had at least 2 smear examinations (the first smear being negative), and the number first identified only from the third smear among all those who had 3 smears examined (the first 2 being negative). The determination of these proportions allowed the estimation of the number of cases missed in those instances where only one or 2 smear examinations had been made and found to be negative, assuming these suspects had an equal probability of being positive at the missed examinations as those who had them actually done (see Appendix).

Results

Thirty-two laboratories were enrolled in the study, in the 8 regions. The results of examination of 61 580 tuberculosis suspects were analysed (Table 1). Among these, 11 650 had at least one examination positive for acid-fast bacilli. A total of 141 371 smear examinations

Address for correspondence: Dr Hans L. Rieder, Chief, Tuberculosis Section of IUATLD, Jetzikofenstrasse 12, 3038 Bern, Switzerland; fax +41 31 829 4576.

Table 1. Summary of activities of the selected laboratories in 8 regions of Tanzania

Region	Tuberculosis: No. of suspects	Tuberculosis: No. of cases	Smears: No. made	Smears: No. per working day	Smears: No. per case
Iringa	5065	880 (17·4%)	12269	4·5	13·9
Kigoma	3389	486 (14·3%)	8090	5·5	16·6
Lindi	5343	1029 (19·3%)	12092	3·4	11·8
Mara	10000	1748 (17·5%)	25538	7·1	14·6
Mtwara	4964	1183 (23·8%)	13240	11·1	11·2
Mwanza	21932	3963 (18·1%)	45357	8·9	11·4
Singida	5447	1289 (23·7%)	12110	3·7	9·4
Tanga	5440	1072 (19·7%)	12675	13·3	11·8
Total	61580	11650 (18·9%)	141371	6·5	12·1

Table 2. Pattern of smear results among new tuberculosis suspects in 8 regions of Tanzania

Region	No. of negative smears: Smear 1[a]	Smears 1, 2[b]	Smears 1, 2, 3[c]	No. of positive cases[d]: Smear 1	Smear 2	Smear 3
Iringa	746	743	2696	803	62	15
Kigoma	542	778	1583	426	42	18
Lindi	1196	785	2333	907	101	21
Mara	1251	1461	5540	1466	208	74
Mtwara	430	413	2938	1022	108	53
Mwanza	6706	3911	7352	3534	359	70
Singida	1225	960	1973	1192	82	15
Tanga	1119	844	2405	926	123	23
Total	13215	9895	26820	10276	1085	289

[a]1st smear negative, 2nd and 3rd smears not done.
[b]1st and 2nd smears negative, 3rd smear not done.
[c]All 3 smears negative.
[d]No. of cases first positive on smear indicated.

Table 3. Observed and expected tuberculosis cases from serial smear examinations in 8 regions of Tanzania

Region	No. of cases found	No. of cases missed[a]	Total no. of cases expected	Percentage expected on: First smear	Second smear only[b]	Third smear only[b]
Iringa	880	17·8	897·8	89·4	8·2	2·4
Kigoma	486	24·8	510·8	83·4	10·6	6·3
Lindi	1029	74·9	1103·9	82·2	13·4	4·5
Mara	1748	68·6	1816·6	80·7	13·3	6·0
Mtwara	1183	27·2	1210·2	84·4	10·2	5·4
Mwanza	3963	335·6	4298·6	82·2	13·4	4·4
Singida	1289	50·4	1339·4	89·0	8·7	2·3
Tanga	1072	70·9	1142·9	81·0	14·7	4·2
Total	11650	670·2	12320·2	83·4	12·2	4·4

[a]Based upon estimates of yield for consecutive examinations of those with incomplete successive examinations (see Appendix).
[b]Not detected on previous smear(s).

was performed on these suspects, requiring an average of 6·5 smears per working day in the 8 regions; on average, 12·1 smears were examined to detect each individual case.

Of the 49 930 tuberculosis suspects with negative results, 26·5% had a single negative examination, 19·8% had 2 negative examinations, and only 53·7% had 3 negative examinations (Table 2). Of the 11 650 cases identified, 88·2% were found for the first time on the first examination, 9·3% for the first time on the second, and 2·5% for the first time on the third.

Because only about half of all suspects had 3 examinations done, the number of cases missed by failing to do a second or third examination among negative cases was calculated (see Appendix) to be 670·2 (5·4% of the expected total) (Table 3).

Taking the expected number of cases as the denominator, the incremental yield of each smear was calculated (Table 3), showing that the first smear was expected to yield 83·4%, the second 12·2%, and the third 4·4% of all expected cases.

The variation in results by laboratory-year is depicted in the Figure. The median incremental yield was 85·6% for the first smear examination, 11·2% for the second, and 4·6% for the third.

Discussion

Information provided by laboratory registers is important for management of supplies, because the programmes must base the amount of laboratory material required on the number of notified cases. The recommendation of the IUATLD for the calculation of sup-

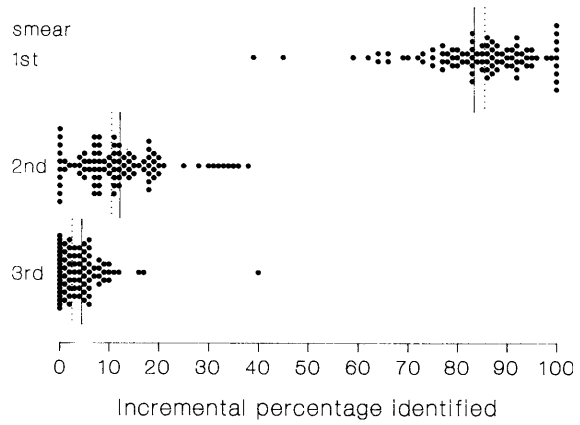

Figure. Expected incremental yield from serial sputum smear examination for acid-fast bacilli in 94 laboratory-years in 8 regions of Tanzania. Each point represents the results from a single laboratory in a single year; continuous vertical lines indicate the means, and dotted vertical lines show the medians.

plies in laboratory material has been that 10 suspects need to be examined with 3 smears each to identify one case of sputum smear-positive tuberculosis (ENARSON *et al.* 1994; RIEDER & ENARSON, 1995). The basis of the calculation is clearly generous in the setting of Tanzania, where the proportion of cases among suspects was close to 20% and the number of smears actually examined was 12 per new case. Similar evaluations may be useful in other settings where the criteria defined to examine a suspect and the prevalence of other conditions meeting these criteria may vary from those in this study.

The specificity of microscopical examination for acid-fast bacilli in identifying *M. tuberculosis* is determined by the ratio of the prevalence of *M. tuberculosis* complex to that of environmental mycobacteria in clinical specimens, and the extent of technical errors in the laboratory. Positive sputum smears due to environmental mycobacteria are very uncommon in many sub-Saharan African countries (ABER *et al.*, 1980; BRAUN *et al.*, 1992). The false positive results due to technical errors are reduced by requiring at least 2 positive smears to classify a patient as having sputum smear-positive tuberculosis (WHO, 1991). This study was not designed to evaluate the specificity of smear examination and only a single positive smear was required for study purposes to define a case.

The sensitivity of sputum smear examination is of major interest in most low income countries, where it is the only practical method of bacteriological confirmation of tuberculosis. Even a single sputum smear examination detects a high proportion of all patients from whom *M. tuberculosis* is ultimately isolated in a culture of the same specimen, as shown in a study in India in the 1950s (ANDREWS & RADHAKRISHNA, 1959) in which microscopical examination of sequential specimens from the same patient was capable of detecting most cases subsequently positive on sputum cultures: 76·7% from the first specimen, 83·0% from the second, 83·9% from the third, and 85·3% from the fourth. The incremental return on specimens subsequent to the second was thus very small. These results led to a recommendation that, in many low income countries, examination of 2 consecutive sputum smears is almost as efficient as culture at detecting cases (MITCHISON, 1968).

The question of the incremental yield of acid-fast bacilli from serial examinations is the next to address. In a study in Singapore (CHAN *et al.*, 1971), of those smear positive on at least one specimen, 78·6% were positive on both specimens, 7·7% only on the initial specimen, and 13·7% only on the second. In the study in India (ANDREWS & RADHAKRISHNA, 1959), 77·4% were detected on an initial spot specimen, 89·9% on an initial collection specimen, 95·5% after one spot and one collection specimen, and 98·3% after 3 specimens—spot–collection–spot. The standard recommendation, to obtain 3 sputum smears, the first on the spot, the second in the early morning, and the third again on the spot when the second is delivered (ENARSON *et al.*, 1994), has been implemented in Tanzania only recently. Most laboratories were collecting 3 successive early morning specimens at the time of the study.

The incremental yield of sputum smear microscopy in industrialized countries is remarkably similar to that observed in low income countries. In a study in the laboratory of a large medical facility in Colorado, USA, from 1968 to 1973, BLAIR and co-workers (1976) found that, if 6 consecutive smears were made, the incremental gain was 9·0% from the first to the second smear, 7·8% from the second to the third, and about 2% on each of the following 3. Several other studies have shown remarkably similar results to those presented here, with an 80–82% yield from the first, a 10–14% from the second, and 5–8% from the third smear examination (URBANCZIK, 1985).

Tanzania has a case notification rate of sputum smear-positive pulmonary tuberculosis substantially lower than that of either India in the 1950s or Singapore in 1969, and higher than that of the USA in the late 1960s (ENARSON *et al.*, 1993), but which is continuously rising due to the impact of the epidemic of human immunodeficiency virus. The similarity of results, in spite of these differences in setting, confirms the appropriateness of the current recommendations in terms of the benefits obtained, but also indicates that routine microscopical examination of 2 consecutive specimens of sputum gives a yield of cases sufficiently high to be the basis of case-finding in low income countries, should the work-load dictate a reduction in the number of examinations.

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References

Aber, V. R., Allen, B. W., Mitchison, D. A., Ayuma, P., Edwards, E. A. & Keyes, A. B. (1980). Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. *Tubercle*, **61**, 123–133.

Andrews, R. H. & Radhakrishna, S. (1959). A comparison of two methods of sputum collection in the diagnosis of pulmonary tuberculosis. *Tubercle*, **40**, 155–162.

Blair, E. B., Brown, G. L. & Tull, A. H. (1976). Computer files and analyses of laboratory data from tuberculosis patients. II. Analyses of six years' data on sputum specimens. *American Review of Respiratory Diseases*, **113**, 427–432.

Braun, M. M., Kilburn, J. O., Smithwick, R. W., Coulibaly, I. M., Coulibaly, D., Silcox, V. A., Gnaore, E., Adjorlolo, G. & De Cock, K. M. (1992). HIV infection and primary resistance to antituberculosis drugs in Abidjan, Côte d'Ivoire. *AIDS*, **6**, 1327–1330.

Chan, W., Chia, M., Lee, L. K. & Macfadyen, D. M. (1971). Bacteriological measures for the detection of cases of pulmonary tuberculosis. *Bulletin of the World Health Organization*, **45**, 551–558.

Debrunner, M., Salfinger, M., Brändli, O. & van Graevenitz, A. (1992). Epidemiology and clinical significance of nontuberculous mycobacteria in patients negative for human immunodeficiency virus in Switzerland. *Clinical Infectious Diseases*, **15**, 330–345.

Enarson, D. A., Chum, H. J., Gninafon, M., Nyangulu, D. S. & Salomao, M. A. (1993). Tuberculosis and human immunodeficiency virus infection in Africa. In: *Tuberculosis*, Reichman, L. B. & Hershfield, E. S. (editors). New York: Marcel Dekker, pp. 395–412.

Enarson, D. A., Rieder, H. L. & Arnadottir, T. (1994). *Tuberculosis Guide for Low Income Countries*, 3rd edition. Paris: Inter-

national Union Against Tuberculosis and Lung Disease.
Grzybowski, S., Barnett, G. D. & Styblo, K. (1975). Contacts of active pulmonary tuberculosis. Tuberculosis Surveillance Research Unit report no. 3. *Bulletin of the International Union Against Tuberculosis*, **50**, 90–106.
Mitchison, D. A. (1968). Examination of sputum by smear and culture in case-finding. *Bulletin of the International Union Against Tuberculosis*, **41**, 139–147.
Rieder, H. L. & Enarson, D. A. (1995). A computer-based ordering system for supplies in national tuberculosis programs. *Tubercle and Lung Disease*, **76**, 450–454.
Shaw, J. B. & Wynn-Williams, N. (1954). Infectivity of pulmonary tuberculosis in relation to sputum status. *American Review of Tuberculosis*, **69**, 724–732.
Stýblo, K., Daňková, D., Drápela, J., Galliová, J., Ježek, Z., Krivánek, J., Kubík, A., Langerová, M. & Radkovsky, J. (1967). Epidemiological and clinical study of tuberculosis in the District of Kolin, Czechoslovakia. Report of the first 4 years of the study (1961–64). *Bulletin of the World Health Organization*, **37**, 819–874.
Tanzania (1987). *Manual of the National Tuberculosis/Leprosy Programme in Tanzania for the District Tuberculosis/Leprosy Coordinators*, 2nd edition. Dar es Salaam: Ministry of Health.
Toman, K. (1979). *Tuberculosis — Case-finding and Chemotherapy. Questions and Answers*. Geneva: World Health Organization.
Urbanczik, R. (1985). Present position of microscopy and of culture in diagnostic mycobacteriology. *Zentralblatt für Bakteriologie und Hygiene*, **260**, 81–87.
Van Geuns, H. A., Meijer, J. & Stýblo, K. (1975). Results of contact examination in Rotterdam, 1967–1969. Tuberculosis Surveillance Research Unit report no. 3. *Bulletin of the International Union Against Tuberculosis*, **50**, 107–121.
WHO (1991). *Guidelines for tuberculosis treatment in adults and children in national tuberculosis programmes*. Geneva: World Health Organization, mimeographed document, WHO/TUB/91.161.
Yaiko, D. M., Nassos, P. S., Sanders, C. A., Madej, J. J. & Hadley, W. K. (1994). High predictive value of the acid-fast smear for *Mycobacterium tuberculosis* despite the high prevalence of *Mycobacterium avium* complex in respiratory specimens. *Clinical Infectious Diseases*, **19**, 334–336.

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Appendix

Formulas to calculate fractions of expected cases found with one, 2, and 3 smears

For the analysis, 6 essential patterns of smear results can be distinguished. 'Essential' is defined here as the point in successive examinations when a smear is positive for the first time. It thus neglects all results of examinations subsequent to the first positive result. The 6 patterns are thus as follows:

n00 denotes 1st smear negative, 2nd smear not done, 3rd smear not done;
nn0 denotes 1st smear negative, 2nd smear negative, 3rd smear not done;
nnn denotes 1st smear negative, 2nd smear negative, 3rd smear negative;
px denotes case positive for the first time on 1st smear;
npx denotes case negative on 1st smear, positive for the first time on 2nd smear;
nnp denotes case negative on 1st smear, negative on 2nd smear, positive for the first time on 3rd smear;

where *x* indicates negative, positive, or not done.

In a first step, the proportion of cases found on the first (s_1), the second (s_2), and the third (s_3) smear are determined:

$$s_1 = \frac{px}{n00 + nn0 + nnn + px + npx + nnp}$$

$$s_2 = \frac{npx}{nn0 + nnn + npx + nnp}$$

$$s_3 = \frac{nnp}{nnn + nnp}$$

Assuming that subjects with one negative smear, but no subsequent examination, and subjects with 2 negative smears, but no subsequent examination, are as likely to be positive on the second or third smear respectively as those who actually had 2 or 3 smears examined, the number of cases missed by failing to do a second (m_2) and/or third (m_3) smear respectively can be calculated:

$$m_2 = s_2 \times n00$$

In order to calculate the expected number of positives missed by failing to examine a third smear, both those having 2 negative smears but no third smear and those having only one negative smear who would be expected to have a negative second smear must be considered. From the above equation the number of subjects having only one negative smear who would be expected to have a negative second smear is:

$(1 - s_2) \times n00$, thus $m_3 = s_3\,(nn0 + (1 - s_2) \times nn0)$

The total number of cases missed (M) is: $M = m_2 + m_3$

The total number of expected cases (E) is thus: $E = M + px + npx + nnp$

Using the number of expected cases as the denominator, the incremental fractions found on the first (f_1), second (f_2), and third (f_3) smear are:

$$f_1 = \frac{px}{E}$$

$$f_2 = \frac{npx + m_2}{E} = \frac{npx + s_2 n00}{E}$$

$$f_3 = \frac{nnp + m_3}{E} = \frac{nnp + s_3(nn0 + (1 - s_2) \times n00)}{E}$$