Comparison of Tuberculosis Case-Finding in a High Prevalence Area

C. DE VILLE DE GOYET, H. H. KLEEBERG

SUMMARY

This comparison of bacteriological methods for tuberculosis case-finding was made on 1 595 specimens from persons in 16 locations in the Transkei. Sputum induced by mechanical stimulation of epiglottis and larynx was superior to cultured laryngeal swabs. The 2-minute examinations by fluorescence microscopy yielded 1,5 times as many positive smears as 4-minute examinations by light microscopy. Culture yielded 97 strains of *M. tuberculosis*, or 6,6%, and 171 colonies of other mycobacteria. Of the tuberculous sputa 58% had only 20 colonies or less and none of these were recognised to contain tubercle bacilli by microscopy. Of the culture-positive smears 36% had been diagnosed on smear. The potential yield of inoculating only one slope was calculated to be 73% of the positive specimens. For high prevalence areas such as the Transkei a network of facilities for microscopic examination is recommended.

S. Afr. Med. J., **48**, 2582 (1974).

Tuberculosis control programmes vary according to the prevalence of the disease, rural or urban environment, availability of health services and laboratories, and other conditions, such as the accessibility and the development of the people. In South Africa the Transkei is a high prevalence region with many special problems. The data originate from a field study of bacteriological case-finding undertaken in 16 randomly selected locations in the Transkei in close co-operation with the South African National Tuberculosis Association and the local State health services. The data are published in detail in another article. They are analysed here to determine the relative efficiency of microscopy and culture for case-finding, and the importance of various technical and operational factors.

PATIENTS AND METHODS

Population, Interrogation and Recording

At the 16 locations 1 595 persons aged 15 years or older, were investigated. A preponderance of females and elders was noticed at every site because the able-bodied Black men migrate to the towns and industrialised regions to seek work. Control of tuberculosis among the migratory men is usually very good. The sample obtained may be considered as representative of the population wanted for examination by the mobile teams and at which the campaign was aimed.

Each person was carefully questioned in Xhosa by Black nurses or health assistants about previous or current anti-TB treatment, and about the presence of TB-like symptoms, such as lasting cough, blood in sputum, loss of appetite, pain in chest, sweating during night, and shortage of breath. Records were made on the spot on a printed card. No medical examination was performed. The benefit of restricting case-finding to the symptomatic persons is discussed in another report.[1]

Collection and Transportation of Specimens

One bottle and 2 swabs were provided for each person. Collection started early in the morning at the headman's dwelling or at the school of the location. With the assistance of a laryngoscope a 15-cm long wooden swab was introduced to irritate the epiglottis and larynx thus producing a forced cough and good sputum. Without this procedure the majority of specimen bottles would have been empty. A second swab was then used to collect sputum inside the mouth. Face masks were worn for protection of the operator. Between 50 and 300 samples were taken per location, depending on the size of the location. Samples were transported on ice to the nearest airport, and flown to Pretoria. They were processed the next morning, usually within 24 hours. Not all the sputa were satisfactory as to quality and quantity. This was related to care taken by the collecting doctors.

Cultures and Smears

After transfer into centrifuge tubes the sputum was mixed with equal parts of 4% NaOH. It was shaken in a heated waterbath (37°C) for 15 minutes, and then spun down in a centrifuge for a further 15 minutes. The supernatant was discarded and the sediment neutralised. Löwenstein-Jensen medium in 3 McCartney bottles was inoculated by loop, and the same loop was used to prepare 2 smears on 2 slides each. To prevent washing off of the material during staining, slides were alcohol-cleaned and a drop of serum was mixed with the sputum concentrate before fixation. Smears were heat-fixed, treated and stained in 2 ways—one by the Ziehl-Neelsen method, and the other for fluorescence microscopy by the auramine/rhodamine method. The ingredients were as follows: auramine 1,5 g,

Tuberculosis Research Unit of the South African Medical Research Council, Onderstepoort, Tvl
C. DE VILLE DE GOYET
H. H. KLEEBERG

Date received: 27 December 1973.

rhodamine 0,75 g, glycerine 75,0 ml phenol 10,0 ml, distilled H_2O 50,0 ml. Decolourisation was done with acid alcohol: 0,5% HCl in 70% ethyl alcohol; counterstaining with 0,5% potassium permanganate solution. First staining lasted 15 minutes, decolourisation 2 minutes, counterstaining ½ minute.

All smears were examined by a trained microscopist, who screened the smears for 4 minutes if negative on light microscopy and for 2 minutes for fluorescence microscopy.

The cultures were incubated for 8 weeks, and read each week for 4 weeks and then after 8 weeks (90% of the positive cultures were visible after 4 weeks). Colonies were counted if possible, and when they were atypical in appearance this was specially noted. If growth was abundant, drug sensitivity tests were made immediately, otherwise a subculture was made to produce more growth for identification and drug sensitivity testing. If there was any doubt about the genus of the colony, it was microscopically investigated for acid-fastness and shape by an experienced bacteriologist. For confirmation of species identification the niacin tests on all non-pigmented strains, and a test for pyromucic acid hydrazide sensitivity were made.

Drug sensitivity tests would normally not be undertaken in a mass campaign, but in this case we wished to check any initial drug resistance in that area for isoniazid, streptomycin, PAS, thiacetazone and ethionamide. The results of these tests, and all findings on mycobacteria of other species will be presented in other articles.

RESULTS AND DISCUSSION

The various bacteriological findings are summarised in Table I. Ordinary light microscopy after Ziehl-Neelsen staining was positive in 1,7% of smears, compared with 2,1% of positive smears by fluorescence microscopy and 6,6% of positive samples on culture. Cultures made from the swabs yielded growth in 0,2% of cases only.

Performance of Two Microscopical Techniques

Of the 1 595 sputa, 1 227 were satisfactorily stained by both techniques. Since 1 trained microscopist read all smears, a delay of 2 months occurred, but such a delay does not influence the sensitivity of either method.[2,3] The percentage of smears which were unsatisfactory because the material had washed off was very similar for both methods; it was disregarded for analysis. The performance of the methods (compared in Table II) is in favour of fluorescence microscopy with 18 (1,5%) positive after Ziehl-Neelsen staining and 27 (2,2%) positive after staining with auramine/rhodamine. Several authors have reported similar findings.[4-8]

Rao and Nagpaul[5] demonstrated that the sensitivity of direct microscopy varies with different proportions of highly positive to scantily positive sputa. A similar finding is seen in Table II. Of 9 specimens which were negative under ordinary light microscopy, 8 were found to be scantily positive under fluorescence microscopy. The greater sensitivity of the latter method is most useful for diagnosing scantily positive sputa. In our study, 2 smears were made of each sputum, while 1 slide was successively examined by both methods by others.[6,7] Greater efficiency, higher sensitivity and saving of time was also seen by Mitchison,[7-10] but fluorescence microscopes are at least twice as costly and necessitate extensive training of microscopists.

TABLE I. SUMMARY OF THE BACTERIOLOGICAL RESULTS — TRANSKEI BACTERIOLOGICAL SURVEY

Method	Tests completed	Positive			
		M. tuberculosis		Other mycobacteria	
		No.	%	No.	%
Ziehl-Neelsen	1 328	22	1,7	1	0,1
Fluorescence	1 354	28	2,1	4	0,3
Sputum culture	1 546	93	6,0	171	11,0
Laryngeal swab culture	1 249	3	0,2	5	0,4
Total cultures	2 795				

TABLE II. COMPARATIVE YIELD OF TWO TECHNIQUES OF DIRECT MICROSCOPICAL EXAMINATION

Ziehl-Neelsen		Fluorescence				Total	
		Negative	Scanty	Moderate	Numerous	No.	%
Negative		1 200	8	—	1	1 209	98,5
Scanty		—	2	5	—	7	0,6
Moderate		—	—	—	2	2	0,2
Numerous		—	—	—	9	9	0,7
Total	No.	1 200	10	5	12	1 227	
	%	97,8	0,8	0,4	1,0		

TABLE III. POSITIVITY OF SPUTUM CULTURE VERSUS NUMBER OF POSITIVE SLANTS

No. of positive slants		Degree of positivity for *M. tuberculosis*: 1 - 5 colonies No	%	6 - 20 colonies No.	%	+ or ++ No.	%	+++ or ++++ No.	%	Total sputa
1	No.	31	91,2	2	5,9	1	3,0	—	—	34
	%	70,5		20,0		5,9		—	—	36,6
2	No.	8	89,0	1	11,0	—	—	—	—	9
	%	18,2		10,0		—		—		9,7
3	No.	5	10,0	7	14,0	16	32,0	22	44,0	50
	%	11,4		70,0		94,1		100,0		53,8
Total sputa		44	47,3	10	10,8	17	18,3	22	23,7	93

Key: Percentages to the right of the figure relate to totals in the horizontal, percentages below the figure relate to the vertical.

TABLE IV. RESULTS OF SPUTUM CULTURE CORRELATED TO THE NUMBER OF TUBES CONTAMINATED OR DRIED OUT (1 595 SPECIMENS)

No. of unreadable tubes	No. of specimens	Negative	*M. tuberculosis*	Other mycobacteria
0	1 379	1 152	86	141
1	142	106	7	29
2	25	24	—	1
3	49	No diagnosis	No diagnosis	No diagnosis

Culture Results Related to Number of Slants

Each of the 1 595 sputa were inoculated onto 3 slants of Löwenstein-Jensen medium. Of these, 49 specimens were heavily contaminated on all 3 slants, or the medium dried out. In Table III the specimens yielding growth of *M. tuberculosis* are correlated to the number of positive slants. Fifty specimens (5,38%) were positive on all 3 bottles, including 38 of the 39 sputa which were significantly positive, i.e. + to ++++.

An over-all loss of 6% through contamination is not alarming in a field survey. The people held the open bottles for a minute or two in their unclean hands in the open field, and some also had foreign matter in their mouths. The distribution of the number of contaminated slants is shown in Table IV (1st and 2nd columns). Contamination of a single tube (142 cases) may in fact have been caused inside the laboratory. Should the contamination of a slope be an independent random phenomenon, the number of specimens in column 2 of Table IV would read 1 322, 254, 16 and 3, respectively, and the loss of a sample would be a very rare occurrence: 3 out of 1 595.

The potential yield of inoculating 2 and 1 slope respectively is shown in Table V. Out of 93 positive specimens, only 68 (73,2%) would have been found had the specimen been inoculated onto a single bottle. The number of specimens actually not evaluated because of contamination would have risen from 49 (3,1%) for 3, to 57 (3,6%) for 2, and 113 (7,1%) for 1 single media slope. In a case-finding programme the loss of some low-yield cultures (1 - 5 colonies) is offset by saving in time and increase of capacity of the laboratory. In an epidemiological survey this loss of some positives due to restriction in media would lead to an underestimation of the prevalence rate of carriers of *M. tuberculosis* and of other mycobacteria.

TABLE Va. OBSERVED AND CALCULATED CONTAMINATION RATES FROM 1 595 SPECIMENS

No. of slants inoculated	No. of contaminated slants: 1	2	3
3*	142	25	49
2†	112	57	NA
1†	113	NA	NA

TABLE Vb. OBSERVED AND CALCULATED YIELD OF CULTURING OF 1 595 SPUTA

No. of slants inoculated	No. of positive slants (*M. tuberculosis*): 1	2	3	Total
3*	34	9	50	93
2†	29	53	NA	82
1†	68	NA	NA	68

* Observed values.
† Values calculated by combinational analysis.

TABLE VI. COMPARATIVE YIELD OF SPUTUM EXAMINATION BY FLUORESCENCE AND CULTURE
(M. TUBERCULOSIS)

Fluorescence microscopy	Sputum culture: Negative	1 - 5 col.	6 - 20 col.	+ or ++	+++ or ++++	Total: No.	Total: %
Negative	1 095	35	9	2	1	1 142	97,5
Scanty	2	—	—	7	—	9	0,8
Moderate	—	—	—	3	2	5	0,4
Numerous	—	—	—	1	14	15	1,3
Total No.	1 097	35	9	13	17	1 171	
Total %	93,7	3,0	0,8	1,1	1,5		

TABLE VII. PROPORTION OF CULTURE-POSITIVE SPUTA CONFIRMED BY DIRECT SMEAR EXAMINATION

	Year	Source of sputa	Microscope method	Cases pos. by culture	Positive by smear and culture: No.	Positive by smear and culture: %	Collection
London[7]	1954-62	Newly diagnosed patients	F	773	272	35,2	Hospital
France[8]	1965-69	Newly admitted or readmitted patients	F	761	618	81,2*	Hospital
France[10] (Lille)		Routine specimens	F	1 861	900	48,4	
India[1]	1961-63	Abnormal X-ray shadow	F	326	149	45,7	Field survey
India[2]	1962-63	Symptoms suggestive of PTB	F	182	121	66,5	Case-finding
India[3]	1963-64	Symptoms suggestive of PTB	F	129	106	82,2	Clinics
India[3]	1962-63	Treated cases	F	59	51	86,4	Clinics
		Untreated cases	F	102	87	95,1	Clinics
India[4]	1956-58	Newly diagnosed patients	F	324	267	82,4	Clinics
Singapore[5]	1969	Newly admitted cases (X-ray findings consistent with PTB)	F	406	321	87,0	Clinics
Thailand[11]	1960-65	X-ray abnormalities	ZN	157	56	35,7	Field survey
Germiston	1965-1972	Routine specimens (hospital patients)	ZN	5 700	4 314	75,7	Hospital
Cape Town[9]	1972	Newly-admitted cases (X-ray findings consistent with PTB)	ZN	80	49†	61,3	Hospital
			ZN	80	62‡	77,5	Hospital

F = fluorescence method; ZN = Ziehl-Neelsen method
* = Repeated specimens.
† = 1 specimen.
‡ = 3 specimens.

Yield of Microscopy Compared with Culture

Since the fluorescence method was more efficient than the Ziehl-Neelsen microscopy, the results of the latter will not be further considered here. In all, 1 171 specimens were examined by both sputum culture and fluorescence microscopy and the relative yields are shown in Table VI. Of the 74 culture-positive specimens 27 (36,5%) were positive also by fluorescence microscopy. The greater sensitivity of culture compared with microscopy is generally accepted. According to a review by Bartmann *et al.*[10] the failure rate of microscopy varied between 23% and 48%. At the Knights Laboratory at Germiston 2 000 comparative tests were done annually over many years, and culture consistently yielded about 25% more cases than microscopy, the sputum originating from treated patients. Table VI also shows that none of the 44 samples with low or single colony counts was positive under the microscope.

The various degrees of positivity, the viability of tubercle bacilli and the refinement of the various techniques are influencing factors, as may be seen from Table VI. All but 2 of the microscopically positive sputa produced more than 20 colonies on culture. None of the positive cultures with less than 20 colonies of *M. tuberculosis* was indicated by microscopy. This feature partly explains the different rates observed in Table VII.

In conditions similar to ours, as in the field surveys in India and Thailand, the proportion of positive culture cases detected microscopically was less than 50%. In epidemiological studies comparatively more cultures with few colonies are encountered. In subjects with symptoms

suggestive of pulmonary tuberculosis, the proportion of significantly positive sputa, and consequently the proportion of positive microscopy, is larger. According to Mitchison[5] the difference between Madras' and London's respective yields by direct microscopy (Table VII) lay in the severity of the disease. In untreated patients with clinical and/or radiological signs of pulmonary tuberculosis the relative efficacy of smear examination varied between 61% and 95%.

The refinement and improvement of the 2 procedures affect their relative yield. In Cape Town[17] the relative proportion of positive cultures confirmed by direct smear examination increased from 61,3% to 77,5% when microscopy was performed on 3 successive specimens. Various additional factors have been investigated, such as intake of drugs, transport and storage conditions, number of specimens collected, time of collection (overnight or spot specimens), number of slants inoculated for culture, training of microscopical examiner, and time spent on examination.

From Table VI it is clear that 2 specimens were negative on culture and scantily positive by fluorescence.

Such misleading positive smears caused by non-viable bacilli do not occur as frequently in a case-finding programme as they do in the bacteriological assessment of chemotherapy. This problem was well studied by Parrot *et al.*[18] The influence of bacteriostatic drugs must be considered when positive microscopical results conflict with negative cultures.

False positives are more frequent among smears with very few bacilli. This situation is encountered mostly in prevalence surveys and mass bacteriological findings. Other authors recommend the reporting of a positive result only when a smear has at least 5 - 10 bacillary forms.[5,8] Repetition of specimen collection greatly reduces the number of false positives on microscopy.[10] In a mass case-finding this procedure would be too costly.

Laryngeal Swab Culturing

Coughing and expectoration were induced by tickling the individual's larynx with 2 sterile laryngeal swabs. This procedure was successful in producing cough, (plastic flexible rods are preferable to wooden sticks which tend to break).

Culturing of the swab material was done for the first 1 249 specimens with a very low total yield of 0,2% (Table I). Only 8 specimens grew in the fluid Kirschner medium. Those positive for *M. tuberculosis* were also significantly positive on the sputum culture. The 5 cultures corresponding to the 5 swab cultures with other species of mycobacteria did not grow.

Laryngeal swab-taking improved the amount and quality of sputum and thereby the yield of sputum culturing, but nothing was gained by actually culturing the swabs in this case-finding programme.

Epidemiological and Economic Considerations

Since resources are always limited they must be allocated in the optimal way. To cut the chain of transmission the detection of infectious cases must obviously be given priority in a control programme. Raj Nairain *et al.*[19] found the rate of infection among children aged 0 - 14 years to be 41%, 20% and 12% respectively, in households with confirmed cases, suspected cases and no cases of tuberculosis. Similarly, the prevalence of active tuberculosis among 1 116 close contacts of patients with pulmonary tuberculosis was shown to be closely correlated to the bacillary content of the sputum: the prevalence was 6,5% when the sputum was microscopically positive and 1,3% when the bacilli from the index case were found on culture only.[20]

The cost of diagnosing a case by direct microscopy or culture was significantly lower than the cost of finding a case by 70-mm X-ray mobile unit. In the case-finding conditions of the Bantu homelands, where the prevalence of pulmonary tuberculosis is high,[1,18] the unit cost of diagnosing a case bacteriologically is likely to be low.

CONCLUSION

The most direct attack on tuberculosis in an area such as the Transkei is to find the infectious sources and make them non-infectious by treatment. Finding the infectious source requires demonstration by microscopy or by culture, or both. Apart from its low cost in time, money and staff, the great advantage of diagnosis by direct microscopy is that it detects the major part of the total pool of highly positive excretors. Outpatient treatment can be started immediately. Culture is needed especially for detection of individuals excreting only few bacilli. These people are liable to develop active disease at any time.

As a first priority, a permanent network of facilities for microscopical examination in the Transkei and other high prevalence areas is recommended. A network of locally trained microscopists could detect the major part of the pool of highly positive excretors, but it is of no value unless close supervision is kept and the standard of work is continually upgraded. Control by rereading a random selection of smears is necessary for such a scheme.

Culture is of advantage in cases that are less infectious. The cost of culturing has often been overestimated. Culture on a single medium slant was shown to be efficient under case-finding conditions. Since culturing requires more training of staff and day-to-day direct supervision, it leads to a considerable delay between collection of sputum and receipt of results. Laboratory facilities may be restricted to the urban population in the early stages, or mobile facilities may be required where the attendance at health institutions is difficult owing to distance and terrain. Individuals excreting few bacilli may develop more active disease at any time which makes permanent facilities or repeated mass campaigns a real necessity.

Laryngeal swabs are recommended for inducement of cough, but culturing of swabs appears to be of little interest in a case-finding scheme.

Suspects, i.e. persons with X-ray shadows, are of a lower priority in a community-orientated programme. Costly mass X-ray campaigns and long-term chemotherapy of fibrotic or inactive pulmonary lesions contribute little to the control of the disease, and they can block key personnel for long periods of time.

REFERENCES

1. SA Tuberculosis Study Group (1974): S. Afr. Med. J. **48**, 149.
2. Henneghien, C., Horwath, K. and Ballez, C. (1969): Acta tuberc. Pneumol. belg., 6, 878.
3. Mitchison, D. A. (1966). Tubercle (Lond.), 47, 289.
4. Rao, K. P. and Nagpaul, D. R. (1970): Bull. int. Un. Tuberc., **44**, 67.
5. Raj Nairain, M. S., Subba Rao, P. and Chandrasekhar, P. (1971): Amer. Rev. Resp. Dis., 103, 76).
6. Parrot, R., Augier, J., Deshais, B., Gebusson, C. and Gillet, P. (1970): Rev. Tuberc. (Paris), 30, 257.
7. Lucas, J. E. (1970): S. Afr. J. Med. Lab. Tech., 16, 3.
8. Mitchison, D. A (1968): Bull. int. Un. Tuberc., 41, 139.
9. *Idem* (1972): Brit. Med. J., **1**, 424.
10. Bartmann, K., Schütz, J. and Von Vietinghoff-Scheel, O. E. (1964): Praxis Pneumol., 18, 273.
11. Sutters, B. T. and Brogger, S. A. (1967): Bull. Wld Hlth Org., 36, 837.
12. Rao, P., Nair, S. S., Cobbold, N. and Naganathan, D. (1966): *Ibid*., **34**, 589.
13. Tuberculosis Chemotherapy Centre, Madras (1959): *Ibid*., **21**, 51
14. Chan, W., Chia, M., Lo Kwee Lee and Macfayden, D. M. (1971) *Ibid*., 45, 551.
15. Gernez-Rieux, C., Tacquet, A., Tison, F. and Devulder, B. (1968). Rev. Tuberc. (Paris), 32, 21.
16. Taute, J. F. (1972): S. Afr. Med. J., 46, 1674.
17. Parrot, R., Marchal, G., Deshais, B., Augier, J. and Gillet, P. (1971) Rev. Tuberc. (Paris). 35, 197.
18. Raj Nairain S. S., Ramanatha Rao, G. and Chandrasekhar, P. (1966): Bull. Wld Hlth Org., 34, 639.
19. Grzybowski, S. and Allen, E. A. (1964): Amer. Rev. Resp. Dis., 90, 707.
20. South African Tuberculosis Study Group (1974): In preparation