

Case detection

1. What is the role of case detection in tuberculosis control?¹

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Detection of the most infectious cases of tuberculosis – sputum smear-positive pulmonary cases – by case-finding in patients attending health facilities is an essential component of the control of tuberculosis. Its objective is to identify the sources of infection in the community, that is, individuals who are discharging large numbers of tubercle bacilli. Treatment of those infectious patients rapidly renders them non-infectious, thereby cutting the chain of transmission. A secondary benefit of case detection is to minimize the delay in initiating treatment, thereby increasing the probability of cure (1). If the cases detected cannot be treated effectively – because of lack of drugs, poor organization, or patients’ limited access to treatment services – the activity is of little value. Identification of cases without being able to treat them undermines confidence in the health system and increases the number of persistently infectious cases spreading drug-resistant bacilli. Where new cases are not yet treated satisfactorily and reliably cured, resources and efforts should therefore be concentrated on improving treatment outcomes rather than increasing case detection (2). In addition to patients consulting for symptoms, the main target group for case detection is persons who attend health facilities for any reason and present persistent cough, i.e. cough of more than 2 or 3 weeks’ duration.

In the past, case detection has been based on screening of the community by mass miniature radiography (MMR) – so-called “active case-finding”. However, radiological shadows are not specific to the diagnosis of tuberculosis, and, even in patients with active pulmonary tuberculosis, radiographs do not reliably discriminate infectious patients from other cases who do not represent a major risk to the community. Mass screening is not cost-effective since the specificity of the method for identifying sources of infection is low, many cases arise between rounds of screening, and the individuals detected are often not motivated to complete treatment and are frequently lost (3, 4) (see “What is the role of case detection by periodic mass radiographic examination in tuberculosis control?”, page 72).

¹ Based on the chapter in the previous edition by K. Toman.

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Identification of adults with persistent cough attending health facilities and screening them by examination of sputum smears is more cost-effective than MMR and specifically identifies those who are transmitting tuberculosis. In areas where patients are being reliably cured, community education should be provided so that people are made aware that persistent cough is abnormal, informed where health services are available, and persuaded to consult a health provider promptly for sputum smear examination.

Contacts of smear-positive tuberculosis patients are at high risk of infection and of developing tuberculosis, justifying active case detection in these individuals. Examination of contacts, particularly of contacts of sputum smear-positive patients, is therefore recommended to identify and treat tuberculosis cases and to provide preventive treatment to those at highest risk, such as children and people infected with HIV. Among residents of institutions with a high risk of tuberculosis transmission (such as prisons, shelters for the homeless, and hospitals), evaluation for cough on admission and periodic assessments are useful to detect and treat sources of infection.

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2. What is a case of tuberculosis?¹

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Tuberculosis control aims to reduce the spread of infection. The most efficient method for preventing transmission is identification (through case detection, diagnosis) and cure of the most potent sources of infection – pulmonary tuberculosis patients excreting tubercle bacilli (1). In addition, tuberculosis control aims to cure all forms of the disease in order to reduce mortality and human suffering. For the purpose of tuberculosis control programmes, a “case” is therefore defined as a patient in whom tuberculosis has been confirmed bacteriologically or diagnosed by a clinician (2).

For programme purposes, cases are classified according to the site of the lesions as either pulmonary (with lesions in the lung parenchyma) or extrapulmonary (with lesions elsewhere but not in the lung parenchyma). Pulmonary cases are further classified as either sputum smear-positive or sputum smear-negative (which includes smear result unknown). The positivity of smears depends on the number of tubercle bacilli (see “How many bacilli are present in a sputum specimen found positive by smear microscopy?”, page 11) and correlates with the risk of infecting other individuals and the risk of dying from tuberculosis. Contacts of smear-positive individuals are at much greater risk of being infected with *Mycobacterium tuberculosis* and of developing tuberculosis than contacts of tuberculosis patients positive by culture only (3). In countries where culture of sputum samples is readily available, smear-negative cases can be classified as either definite tuberculosis cases (culture-positive for *M. tuberculosis* complex) or others (culture-negative or unavailable).

On diagnosis, patients are classified for registration according to previous TB treatment as:

- new: without or with less than 1 month of previous treatment;
- relapse: smear- or culture-positive patient previously treated and declared cured or treatment completed;
- failure: sputum smear-positive after 5 months or more of treatment (or after 2 months or more of treatment if initially sputum smear-negative);

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- return after default: return to treatment after interruption of 2 months or more;
- transfer in: patient transferred from another tuberculosis register to continue treatment; and
- other: all cases that do not fit the above definitions (includes chronic, i.e. patients sputum-positive at the end of a re-treatment).

Although smear-negative pulmonary tuberculosis and extrapulmonary cases may also be relapses, failures, or chronic cases, this is rare and should be supported by pathological or bacteriological evidence (2).

For registration, there are six mutually exclusive categories of treatment outcome:

- *cured*: a patient who is sputum smear-negative in the last month of treatment and on at least one previous occasion during treatment;
- *treatment completed*: a patient who completed treatment but does not meet the criteria for cure or failure (or after 2 months or more of treatment if initially sputum smear-negative);
- *treatment failure*: a patient who is sputum smear-positive at 5 months or later during treatment;
- *died*: a patient who dies for any reason during the course of treatment;
- *defaulter*: a patient whose treatment was interrupted for 2 months or more;
- *transfer out*: a patient who has been transferred to another unit and for whom the treatment outcome is not known.

Treatment success is defined as the sum of the patients who are cured and who have completed treatment. In countries where culture is current practice, patients can be classified as cure or failure on the basis of culture results (2).

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3. What is the role of sputum microscopy in patients attending health facilities?

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Sputum microscopy is the most efficient way of identifying sources of tuberculosis infection. The method is used to diagnose tuberculosis in persons with suspected pulmonary disease and to identify sources of infection among persons with cough attending health facilities for any reason. Sputum microscopy is also used to monitor the progress of infectious patients during treatment, including confirmation of cure.

Diagnosis

The diagnostic efficiency of sputum smear examination is discussed in “How reliable is smear microscopy?” (page 14). Smear examination has several operational advantages over culture: the results are available sooner, correlate with infectiousness, and identify both patients at high risk of death from tuberculosis if untreated and patients who require more drugs in the initial treatment regimen because of greater bacterial load.

A proportion of the patients attending health facilities consult a physician because of symptoms suggestive of tuberculosis. It is the responsibility of the physician to suspect tuberculosis in these patients and to perform the appropriate diagnostic tests. In diagnosing infectious pulmonary tuberculosis, smear examination in persons with persistent cough is the most important test. Chest radiography is useful for differential diagnosis of pulmonary disease among patients with negative sputum smears. The timing of the diagnostic procedures will depend on the prevalence of tuberculosis in the community. In areas with a high prevalence of tuberculosis, smear examination should be the initial test. For diagnosis of pulmonary disease in areas with a lower prevalence of tuberculosis, smears and chest radiography may be performed simultaneously, a short course of antibiotics nonspecific for tuberculosis may be given, or a chest radiograph may be used as an auxiliary diagnostic procedure before smears and culture. In any case, individuals with abnormal chest radiographs should be asked to submit several sputum samples for smear examination before pulmonary tuberculosis is diagnosed.

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Infectious pulmonary tuberculosis is often not detected until a late stage, even though the patient may have attended health facilities during the initial stages of the disease. Physicians frequently do not suspect tuberculosis or do not request smear examination in patients with cough, particularly if those patients present with non-respiratory ailments. It is estimated that as many as 5–10% of adults attending outpatient health facilities in developing countries may have a persistent cough of more than 2–3 weeks' duration (1, 2). The proportion of smear-positive pulmonary tuberculosis among these individuals depends on the prevalence of tuberculosis in the community. Systematic identification of adults with persistent cough among outpatients in general health facilities can detect a large proportion of sources of tuberculosis infection (3). This reduces treatment delay and identifies infectious patients who are a risk to the community and to other patients and staff at the health facility. Successful treatment of these patients has a rapid effect on tuberculosis prevalence, mortality (4), and transmission (1).

In heavily used facilities, paramedical or administrative staff should be largely responsible for identification of persons with persistent cough and referral for smear examination. This screening is a public health activity intended only to detect and cure sources of infection, and is additional to diagnostic activities in persons consulting spontaneously. Because the objective is primarily to benefit the community, the procedure must be simple, convenient for the individual, and free of charge, and should not detract from the patient's original purpose in attending the clinic. It is important to record the patient's name and address: if the laboratory detects positive smears the patient must be found immediately and treatment initiated.

Culture is not a priority test for systematic detection of cases. Persons who are positive only on culture are less infectious than those who are also positive to microscopy. Furthermore, culture is more expensive and complex than microscopy, and there is a relatively long delay until the result is available.

The duration of cough chosen by a country as the threshold for recommending smear examination depends on the prevalence of smear-positive tuberculosis, the frequency of attendance at health facilities by the population, and the laboratory resources available. If the prevalence of tuberculosis is very low, there is no role for systematic case detection with smears in adults with cough (low cost-effectiveness and high risk of false-positive results). Attendance at health facilities varies among countries. People in more developed countries consult earlier and more often, and the duration of cough selected as a basis for screening must be shorter; however, this increases the proportion of patients with nonspecific cough and the workload of the laboratory services, and reduces cost-effectiveness. Studies of prevalence of cough among adults attending outpatient health facilities help determine the optimal duration of cough at which to recommend sputum examination under routine conditions (2, 5, 6).

Case detection in outpatients by microscopic examination of sputum can significantly increase the number of sources of infection diagnosed. The number of outpatients investigated, the number of smears for diagnosis, and the number of sources detected are indicators of the case-detection activity. In Peru, for instance, 210 905 smear examinations were carried out in 1990, leading to the identification of 24 023 cases of smear-positive pulmonary tuberculosis. In 1993, 602 000 smears from 332 000 persons were examined and 35 646 cases were identified. By 1999 the number of smear-positive cases had decreased to 24 511 despite an increase in the number of smear examinations to 1 938 201 in 1 085 749 persons (1, 4). The proportion of positive smears is an indirect indicator of the impact of the programme in reducing the prevalence of tuberculosis in the community. The rate of smear positivity in persons with respiratory symptoms in Peru was 18.7% in 1990, 14.3% in 1991, 8.5% in 1993, and 2.7% in 1999. Similarly, in Chile the smear positivity rate fell from more than 10% to less than 2% in two decades. By 1999, Peru was examining approximately 5% of the adult population for tuberculosis by smear microscopy every year (1).

Microscopic examination of sputum smears during and at the end of treatment

Sputum smear microscopy has a fundamental role in monitoring the response to treatment of infectious cases of pulmonary tuberculosis. Smear examination should be performed at the end of the initial phase of treatment; if smears are still positive, the intensive phase should be extended for an additional month. Smears should be examined during and at the end of the continuation phase to confirm cure. The *conversion rate* at 2–3 months (defined as the proportion of initially smear-positive patients with negative smears out of the total who started treatment) is a good operational indicator. It shows the capacity of the programme to maintain patients on treatment, obtain smear samples, and eliminate sources of infection, and it is an early surrogate of the treatment outcome indicator (7). With short-course treatment regimens of high efficacy, smears can be positive at 2–3 months because of dead bacilli in patients with negative cultures. Thus, treatment failure based on positive smear examination is not considered until the fifth month or later (see “How can the progress of treatment be monitored?”, page 250). Negative smears during and at the end of treatment are required to declare a patient cured of tuberculosis.

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4. How many bacilli are present in a sputum specimen found positive by smear microscopy?

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If a smear is properly prepared, the number of bacilli it contains will correlate with the concentration of bacilli in the sputum. This numerical relationship, which has been investigated by many authors (1–4), may be illustrated by the following example.

The amount of sputum on a slide for smear preparation is about 0.01 ml. This is spread over an area of 200 mm² (10 × 20 mm). Since the area of an oil-immersion field seen in the microscope is about 0.02 mm², 10 000 such fields would need to be screened in order to examine the entire smear at a magnification of 1000×, i.e. 100× for the oil-immersion objective lens and 10× for the eyepiece. (The size of a field in fluorescence microscopy is about 15 times as large with an objective of 25× and an eyepiece of 10×.) By examining one length (20 mm) of a smear, some 100–120 microscopic fields are screened, representing about 1% of the smear. The above calculations are for a smear that is 10 × 20 mm; in actual practice smears of 20 × 30 mm are generally used.

Thus, if a sputum specimen contains about 5000 bacilli per ml, the entire smear (if prepared as described) will contain about 50 bacilli. If these 50 bacilli were evenly distributed over the 10 000 fields of the smear, there would be one bacillus in 200 fields. If 100 fields were examined the chance of finding this bacillus would be 50%. To find at least three acid-fast bacilli (AFB), about 600 fields would have to be screened. If 300 fields were examined, the chance of finding three bacilli would also be 50% (5–7).

Furthermore, to find one acid-fast bacillus in every 10 fields (or 10 in 100 fields) would require 1000 such bacilli to be present in the smear (10 000 fields) or 100 000 (10⁵) per ml of sputum (Table 1). To find one acid-fast bacillus per field on the average would require 10⁶ bacilli per ml of sputum (Table 1). Thus, a specimen that is consistently found to be positive would have to contain at least 100 000 AFB per ml.

These estimates are based on the assumption that the bacilli are evenly dispersed throughout the specimen, i.e. that each portion of material taken from the specimen will contain the same number of AFB spread evenly over the entire smear. However,

¹ Deceased.

Table 1
Estimated numbers of acid-fast bacilli in sputum specimens and probable numbers of bacilli in smears (estimated minimum values)

No. of oil-immersion fields per bacillus	No. of bacilli per smear	No. of bacilli per ml of specimen
100	100	10 000
10	1 000	100 000
1	10 000	1 000 000

it is known that bacilli are not evenly dispersed in a specimen, but are frequently found in clumps. Thus, when several samples are taken from a sputum specimen, the number of bacilli will vary from one sample to another. Nevertheless, when special culture techniques were used to compare the number of bacilli in large numbers of samples taken from different sputum specimens, certain important observations were made. In particular, the number of colonies cultured from samples taken from the same specimen varied only within certain limits, not at random (see "How reliable is smear microscopy?," page 14). Likewise, variations in colony counts among samples from different specimens did not occur randomly, but were due to differing concentrations of AFB in the specimens. Thus, in spite of considerable sampling variation, the number of bacilli in the smear corresponds fairly closely to the concentration of bacilli in the sputum (4). Below a certain concentration of bacilli in a sputum specimen, the probability that AFB will be transferred from the specimen to the smear and found by microscopy approaches zero. Although it has been estimated that, with optimal laboratory conditions, a positive smear can be obtained with only 100–1000 organisms per ml (8), a more practical estimate is about 10 000 organisms. While a single smear of sputum has a reported sensitivity of only 22–43%, the detection rate goes up considerably when multiple specimens are examined; for example, when 2–3 smears are examined over 2 days, about 50–70% of patients with active pulmonary tuberculosis will have positive smears (9).

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5. How reliable is smear microscopy?

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To assess the reliability of smear microscopy quantitatively, answers are needed to the following questions:

1. What is the probability of finding acid-fast bacilli (AFB) in smears prepared from specimens containing few, some, or many bacilli?
2. What is the probability of reporting a (false-)positive result for smears from specimens without tubercle bacilli?
3. What is the frequency of agreement between microscopists or laboratories reporting the results for smears prepared from the same specimens?

Table 1 under “How many bacilli are present in a sputum specimen found positive by smear microscopy?” (page 11) supplies part of the answer to the first question. The figures in that table are derived from experimental findings and have been extrapolated on the assumption that bacilli are evenly distributed throughout specimens. Since the bacillary content varies from one sample to another, however, such measurements must be performed on a large number of specimens, taking the results of culture as a yardstick (1). In several studies (2, 3), the bacillary counts of smears were compared with the number of colonies grown on cultures prepared from the same specimen.

In a cooperative study by eight laboratories, it was confirmed that colony counts for samples taken from the same specimen varied from one sample to the next, although these variations were minimal.² The disparity of colony counts between samples from different specimens was due mainly to the variation in the concentration of bacilli in these specimens. It was concluded, therefore, that there is a positive correlation between the concentration of culturable bacilli in the specimens, the number of AFB in the corresponding smears, and the probability of their being identified by microscopy. The results (Table 2) show that the chance of finding AFB in a

¹ Deceased.

² David HL et al. *Sensitivity and specificity of acid-fast microscopy*. Atlanta, GA, United States Department of Health, Education and Welfare, Centers for Disease Control (unpublished document prepared for the WHO Expert Committee on Tuberculosis, Geneva, 1973).

Table 2
Number of acid-fast bacilli observed in smears, concentrations of culturable bacilli in sputum specimens, and probability of positive results^a

No. of bacilli observed	Estimated concentration of bacilli per ml of specimen	Probability of a positive result
0 in 100 or more fields ^b	<1000	<10%
1–2 in 300 fields	5000–10 000	50%
1–9 in 100 fields	about 30 000	80%
1–9 in 10 fields	about 50 000	90%
1–9 per field	about 100 000	96.2%
10 or more per field	about 500 000	99.95%

^a Source: reference 1.

^b Approximately 0.01 ml of homogenized sputum was placed on the slide and spread over an area of about 200 mm². The area of a microscope field under oil immersion and at a magnification of 1000× is 0.02 mm². Thus, a smear would contain about 10 000 such fields (see "How many bacilli are present in a sputum specimen found positive by smear microscopy?", page 11).

smear increases with the concentration of bacilli in the specimen. By plotting the data, a smooth curve is obtained, showing that the 50% probability of finding AFB in the smear occurs at a concentration of about 6000 bacilli per ml. Similar values were reported in earlier studies (2, 3).

In order to crosscheck these findings, David et al. tried to determine the probability of not finding any AFB in the smear for various concentrations of bacilli estimated from viability counts.² They examined 431 specimens in three independent experiments. The concentrations of bacilli ranged from 1500 to 300 000 per ml.

Each microscopist was to examine smears from all specimens obtained from a group of selected patients. Uniformity in the technical procedures of smear preparation and examination in the participating laboratories was ensured by a standard protocol. The investigation was designed in such a way that no microscopist could know the results obtained by any other microscopist or the origin of the specimens, or have access to any other information that might result in bias. The proportions of smears reported as negative are shown in Table 3.

Table 3 shows that the probability of not finding AFB in smears decreases steadily as the concentration of bacilli in the specimen increases. When the concentration exceeds 100 000 organisms per ml, the probability of a negative smear result approaches zero. This confirms earlier findings that smears that were consistently positive, at any examination, had been prepared as a rule from specimens containing 10⁵–10⁶ AFB or more per ml.

However, the use of culture colony counts for the calculation of the bacillary content of sputum has limitations, and it is technically difficult to obtain accurate results with this method. Large numbers of samples need to be examined and a special technique

Table 3

Frequency (probability) of negative results for smears from specimens containing varying concentrations of bacilli estimated by culture (colony counts)^a

Estimated concentration of bacilli per ml of specimen	Experiment no.			Mean (%)
	1	2	3	
	negative results (%)			
1 500	—	85	92	88.5
3 000	84	83	77	81.3
15 000	25	28	6	19.6
30 000	16	30	6	17.3
150 000	0	0	5	1.6
300 000	0	0	0	0.0
No. of smears studied	42	100	289	—

^a Reproduced with minor editorial changes from David HL et al. *Sensitivity and specificity of acid-fast microscopy*. Atlanta, GA, United States Department of Health, Education and Welfare, Centers for Disease Control (unpublished document prepared for the WHO Expert Committee on Tuberculosis, Geneva, 1973).

must be used in order to minimize the technical error occurring when specimens contain a large proportion of bacilli in aggregates. (It is impossible to tell whether a colony on a culture medium has grown from a single bacillus or from a clump of bacilli.) On the other hand, AFB that can be seen under the microscope may not always be able to grow on culture, e.g. because they are dead or nonviable (see "What are the main causes of false-positive and false-negative sputum smears?", page 23). The investigators therefore chose a method that does not depend on culture results.

Since the aim was to measure the reliability (reproducibility of results) of the smear microscopy method, the reports of several proficient microscopists who examined smears from the same specimen were compared. Irrespective of whether a report was right or wrong, the frequency of agreement or disagreement between the microscopists was measured. The smears were read strictly independently, according to a protocol. The experiment was arranged as follows.

Four microscopists read 54 specimens. Four smears (one per microscopist) were prepared from each specimen and examined independently. The four results obtained for each specimen were recorded using the scores: negative, scanty (1–9 bacilli in 100 microscopic fields), or positive (1+, 2+, or 3+). The results for each specimen were compared separately, the result of one microscopist being compared with the results of the other three microscopists in all possible permutations. Thus, 12 results were obtained for each specimen. By this means, it was possible to construct a correlation table (Table 4) showing the frequency of agreement and disagreement between the four microscopists. The total number of comparisons was 648, of which four were not reported.

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Table 4
Frequency of agreement or disagreement between four microscopists^a

Report of one microscopist	Reports of all other microscopists ^b					Total no. of observations	
	Negative	Scanty	1+	2+	3+		
Negative	233	25	8	2	0	268	} 309
Scanty ^c	24	5	1	7	4	41	
1+	8	2	11	18	4	43	} 335
2+	2	8	16	39	50	115	
3+	0	4	4	49	120	177	
Total	267	44	40	115	178	644	
	311		333				

^a Source: David HL et al. *Sensitivity and specificity of acid-fast microscopy*. Atlanta, GA, United States Department of Health, Education and Welfare, Centers for Disease Control (unpublished document prepared for the WHO Expert Committee on Tuberculosis, Geneva, 1973).

^b The figures in the box are the readings reported by any microscopist as positive, i.e. 1+, 2+ or 3+.

^c Defined as 1–9 bacilli in 100 microscopic fields.

Table 4 shows that the highest frequency of agreement was on the extreme scores, i.e. negative and 3+ (all identical results are found on the diagonal line). Furthermore, it may be seen from Table 4 that, when one microscopist reported the result as negative or scanty, in only 22 of 309 instances (7%) did other microscopists report a positive result (1+, 2+, or 3+). In other words, there was agreement between the microscopists in 287 of 309 cases (93%). Likewise, when one microscopist reported a positive result, the probability of agreement with the other microscopists was 311 out of 335 (93%).

The lowest frequency of agreement was on results reported as scanty (see Table 4): when one microscopist reported such a result there was an 88% probability (36 out of 41 instances) that other microscopists would disagree. In 24 out of 41 instances (59%) the result reported by other microscopists was negative. This is in accordance with the findings of another investigation, in which sputum specimens from patients with chest symptoms were negative on culture in 3 out of 4 cases when only 1–2 AFB had been seen on the smear (HG ten Dam, 1976, unpublished observations). The definition of scanty used in this classic study was the finding of 1–2 AFB in a smear; such smears should be repeated.

Regarding the grading of positive results, the data show that agreement declined steeply below the score 3+ (Table 5). According to Table 5, agreement on the scores 1+ and 2+ was quite low: 25% and 34% (see data on the diagonal). Thus the differentiation between score 1+ and score 2+ appears to be rather illusory.

The above-mentioned experiment showed the high reliability (reproducibility) of results. By independent examination of smears prepared from the same specimens,

Table 5
Frequency of agreement or disagreement between four microscopists on the score of positive results (data from Table 4 presented in percentages)

		All other microscopists					Total (%)
		Negative	Scanty	1+	2+	3+	
Report of one microscopist	1+	19	5	25	42	9	100
	2+	2	7	14	34	43	100
	3+	0	2	2	28	68	100

the frequency of agreement between equally proficient microscopists may reach 93%. However, these results were achieved under experimental conditions and with experienced laboratory technicians. The question that arises is, "How does smear microscopy work under field conditions, particularly in peripheral health centres of developing countries?" This question is answered below.

Smear microscopy under field conditions in developing countries

In peripheral health centres, sputum collection, the preparation and staining of smears, and their examination by microscopy are usually performed under suboptimal conditions – often by microscopists with limited experience. This applies to most of the peripheral health centres in rural areas, which are attended by the majority of patients complaining of chest symptoms. As a rule, such patients are offered a sputum examination for diagnosis. The standard of case detection in developing countries therefore depends, in addition to operational factors, largely on the technical performance of smear microscopy.

In order to assess the qualitative performance of sputum examination in rural health institutions, several studies were carried out by the National Tuberculosis Institute, Bangalore, India (4, 5). In a South Indian district where a district tuberculosis programme had been implemented about 6 months before the investigation, the performance of nine randomly selected health centres was analysed. The microscopists at these centres were non-specialized health workers who had been trained for 2–4 weeks in the collection and examination of sputum according to a manual that they had been given. They had received on-the-job training from an experienced laboratory technician, who was also a member of the tuberculosis control team (6, 7). The team was responsible for the implementation and supervision of the programme in the entire district (population 1.5 million).

Method of assessment

In each of the nine centres, one sputum sample was collected from every patient complaining of persistent cough and a smear was prepared and examined immediately

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(spot sample). The slide was then sent, together with the specimen, to the laboratory at the National Tuberculosis Institute, where it was re-examined. The specimen was used to prepare a fresh (duplicate) smear, as well as for culture. The results obtained at the peripheral health centre were then compared with those of the reference laboratory, i.e. the results of:

- re-examination of the smear made at the peripheral centre;
- examination of the duplicate smear; and
- culture examination.

The results – in terms of under- or over-reading – were analysed and tabulated for each health centre separately. The result of culture was taken as the yardstick. Of 1681 specimens, 228 (13.6%) were found to be culture-positive and 1453 (86.4%) culture-negative.

Over-reading of culture-negative specimens

In order to estimate the extent of over-reading by the peripheral health centres, the culture-negative specimens were taken as the standard and were compared with the results of the corresponding smears reported by the peripheral centres and by the reference laboratory (Table 6).

There were 1453 specimens negative by culture, of which 2.6% were reported by the health centre as positive. The same smears were re-examined at the reference lab-

Table 6
Over-reading of smears (prepared from culture-negative specimens) read at the peripheral health centre and at the reference laboratory^a

Centre	Total no. of culture-negative specimens	Read as smear-positive at:	
		peripheral health centre	reference laboratory
A	306	5	4
B	233	8	1
C	159	7	7
D	156	2	2
E	108	12	2
F	111	3	1
G	100	1	1
H	84	0	1
I	196	0	0
Total	1453 (100%)	38 (2.6%)	19 (1.3%)

^a Source: reference 5.

oratory, which reported 1.3% as positive. Thus over-reading was, on average, higher at the peripheral health centres than at the reference laboratory. However, a more detailed analysis shows that this difference was attributable mainly to one centre (E). When this centre was excluded from the analysis, the proportion of over-reading fell to 1.9%. The proportion of over-reading by duplicate smear examination was 1.2%, compared with 1.3% by re-examination (5).

Under-reading of culture-positive specimens

In order to estimate the extent of under-reading at the peripheral health centres, the culture-positive specimens were taken as the standard and were compared with the results of the corresponding smears reported by the peripheral centres and by the reference laboratory (Table 7).

There were 228 specimens positive by culture, of which 87 (38.2%) and 67 (29.4%), respectively, were reported by the peripheral health centres and by the reference laboratory as smear-positive. Thus, under-reading at the peripheral health centre was worse than at the reference laboratory (38.2% and 29.4%, respectively). This difference was caused mainly by the poor performance of two centres (D and H). When these two centres were excluded from the analysis, the degree of under-reading at the peripheral centres and at the reference laboratory was practically the same: 23% and 26%, respectively.

The authors of the study concluded (5) that over-reading by the microscopists of

Table 7
Under-reading of smears (prepared from culture-positive specimens) read at the peripheral health centres and at the reference laboratory^a

Centre	Total no. of culture-positive specimens	Read as smear-positive at:	
		peripheral health centre	reference laboratory
A	101	27	26
B	21	7	8
C	23	7	5
D	22	19	9
E	15	6	6
F	16	5	4
G	15	7	5
H	10	8	3
I	5	1	1
Total	228 (100%)	87 (38.2%)	67 (29.4%)

^a Source: reference 5.

the peripheral health centres was a problem in only one of the nine centres. Additional training, supervision, or other corrective action would rectify the deficiency observed. This also applies to under-reading in two of the centres, where corrective training and proper supervision were needed. Comparison of the results with those obtained in other tuberculosis laboratories in India (8, 9) revealed a similar range of over- and under-reading when culture results were taken as the basis.

The authors also concluded that non-specialized staff of general health institutions are capable of carrying out satisfactory smear microscopy. Taking into consideration the short period of training usually received, it may be expected that, with continuous supervision and corrective retraining, the performance of such microscopists could be maintained at a satisfactory level (see “What are the main causes of false-positive and false-negative sputum smears?”, page 23).

In a similar study reported from Algeria (10), the results of re-examination of smears prepared and read by non-specialized staff at a peripheral health centre and re-read at a central laboratory were comparable. Thus, double reading of 104 smears yielded 95% identical results. Of 86 smears classified as negative by the central laboratory, 2 were read as positive by the peripheral health centre, and of 18 smears read as positive at the central laboratory, 3 were judged to be negative at the peripheral health centre. The authors recommended the use of direct smear microscopy at peripheral health centres under the supervision of a central laboratory. Furthermore, they pointed out that it makes little sense to strive for more refined diagnostic techniques or greater precision as long as the health services remain unable to provide adequate treatment for every case diagnosed – the principal purpose of case detection.

Both field studies have indicated that smear microscopy performed by non-specialized health workers may be reliable. Training can be given, even on the job, by qualified technicians. To achieve a satisfactory level of proficiency, however, retraining of those whose performance is below standard must be ensured. Re-examination of smears and examination of duplicate smears prepared from the same specimens are valuable techniques for the supervision and technical assessment of smear microscopy in peripheral health centres. At a later stage, when culture facilities are introduced, culture should be used primarily to assess diagnosis by direct smear examination and then, if possible, for clinical diagnosis and evaluation of treatment.

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6. What are the main causes of false-positive and false-negative sputum smears?

K. Toman¹

False-positive results

Acid-fast particles other than tubercle bacilli

Occasionally, a sputum specimen or smear may contain particles other than *Mycobacterium tuberculosis* that are acid-fast, i.e. retain their red stain (carbol fuchsin) when treated by the Ziehl–Neelsen method and resist decolorization with acid–alcohol. These red particles sometimes resemble tubercle bacilli. They include certain food particles (e.g. waxes, oils), precipitates, other microorganisms, inorganic materials, and artefacts (1–6).

Food particles. To eliminate food particles, the patient should rinse the mouth with clean water (without using toothpaste or disinfectant) before producing the sputum specimen. It is better if the specimen is produced before breakfast.

Precipitated stains. Although precipitated stains are quite easy to differentiate from acid-fast bacilli, they may hamper reading or occasionally mislead an inexperienced microscopist. They can be removed by filtration of staining solutions. However, it is safer to use freshly prepared solutions, filled into carefully cleaned bottles, rather than stale staining solutions.

Environmental acid-fast bacilli. Acid-fast bacilli occur naturally in soil and water, and may occasionally contaminate a specimen or smear during processing. This can be avoided by using distilled water from scrupulously clean containers.

Non-tuberculous mycobacteria and Nocardia species. These occasionally occur in sputum specimens. When they cause pulmonary disease, they may be present in large numbers.

Spores of Bacillus subtilis. They are very rare, mostly of ovoid shape, and larger than tubercle bacilli.

Yeasts. Yeasts may stain slightly red. After heat fixation, they may break into groups of large granules.

Fibres and pollens. Fibres, including those of wool, cotton, filter paper, and bamboo, usually occur singly, most often in only one microscopic field. The

¹ Deceased.

pollen of certain pine trees is seen as short, coccoid rods occurring rarely in specimens.

Scratches on the slide. Scratches may sometimes retain the red stain and confuse inexperienced microscopists. They are usually seen in parallel rows, are generally longer than AFB, and are undulated. They can be identified easily because they are found in a deeper layer on the slide, below the smear, and disappear when the microscopist focuses on the cells (e.g. leukocytes) in the smear.

Contamination through the transfer of bacilli from one smear to another

Acid-fast bacilli may be transferred accidentally from a positive slide to a negative one when several slides are treated simultaneously in staining or decolorization tanks. This can be avoided by processing each slide separately, e.g. on a rack. Contamination may also occur when the wire loop used for making the smear is not correctly flamed. Contamination from this source can be avoided by using disposable wooden sticks for making smears.

Acid-fast bacilli may also be transferred accidentally when the glass rod or dropper used for placing immersion oil on the slide touches the surface of a positive slide and rubs off some of the material onto the next slide. This can also happen if the oil-immersion lens touches the slide or when blotting paper is used for drying several stained smears consecutively. For these reasons, the oil dropper should not touch the smear – the oil should be allowed to drip freely onto the slide – and the oil-immersion objective should never touch the surface of the slide. Before a new smear is examined, the oil should be wiped off the lens with special lens-cleaning paper or a piece of clean cotton tissue. Blotting paper should not be used at all, or for no more than one slide. Slides should never be used more than once for the detection of AFB.

False-negative results

False-negative results (1–6) may be due to deficiencies in the preparation, staining, or examination of the smear. Proper collection of the specimen and subsequent selection of sputum particles are essential to the preparation of a smear and should receive special attention. Poor quality of the sputum sample is the most common reason for a negative sputum smear in a patient with smear-positive tuberculosis. The most common reasons for false-negative results are described below.

Improper sputum collection

Patients are sometimes not told clearly enough what constitutes a proper sputum specimen and how to produce one. It must be made clear that saliva and nasopharyngeal discharge are unsuitable for examination. Patients should be encouraged to stand and be given time to produce bronchial sputum from “deep in the chest”. They should be asked to take several deep breaths, coughing as hard and as deeply as they can. If repeated attempts fail, tickling of the inner surface of the epiglottis or trachea

with a swab may provoke a vigorous cough with sputum. Other techniques to stimulate the production of sputum, such as aerosol induction, administration of beta-agonists, gastric aspiration, and bronchoscopy, may be required in some patients. Inhalation of a warmed solution of hypertonic (3%) saline administered by nebulizer has been shown to induce production of sufficient material for analysis (7). Specimens produced in the early morning are more likely to be AFB-positive than those produced later in the day. If an early-morning sputum specimen is required, patients should be given a container and instructed to place in it the very first sputum produced in the morning, before breakfast.

Improper storage of sputum specimens and stained smears

Stained smears may lose their staining as a result of exposure of the specimen to direct sunlight, radiation (e.g. ultraviolet light), excessive heat, or long periods (more than a week) of storage in hot and humid conditions (8). However, even after a month of storage in tropical climates, specimens have nearly the same rate of smear positivity; all submitted samples should therefore be examined.

Fluorochrome-stained smears lose their fluorescence with storage.

Failure to select suitable sputum particles for smear preparation

Tubercle bacilli are most likely to be found in small roundish masses (“lentils”) of greenish-grey or yellowish matter of a thick, creamy consistency. (Such masses usually consist of dead caseous tissue discharged from a cavity in the lung.) If the sputum is not treated by a special concentration procedure involving centrifugation, these masses have to be carefully separated from the rest of the sputum and transferred to a slide. They can be seen more easily in the sputum against a dark background.

Improper preparation of smears or staining of slides

False-negative results may also be obtained when:

- too little material has been spread on the slide, so that the smear is too thin;
- the smear is too thick, so that sufficient light cannot pass through it;
- the slide was overheated during fixing of the smear;
- the smear has not been sufficiently fixed and parts of the material have been washed off;
- the staining with carbol fuchsin was too short or was overdone by boiling; or
- the counterstaining was too intense, so that the AFB have been obscured.

Improper examination of the smear

If the microscopic examination of the smear is performed erratically or too briefly, too few fields may be examined. False-negative results may also be obtained if the

examiner is unable to distinguish the red-stained AFB because of colour blindness or other visual problems.

Other reasons for false results

Administrative errors

False results may occasionally be obtained because of administrative errors. Such errors may include:

- misidentification of patients, misspelling of names, or confusion of names or of code numbers on specimens and slides;
- mistakes in labelling containers (e.g. writing the identification on the lid instead of the side of the container); and
- falsification of recording or reporting.

Reading errors

Reader or observer error occurs in practically all diagnostic clinical and laboratory work. The nature of this phenomenon, often referred to as the “human factor”, is to a large extent unknown. Nevertheless, under certain conditions, it is measurable. The degree and frequency of errors – over-reading as well as under-reading – vary from one person to another and also in the same individual at different times.

Inter-individual reader variation in smear microscopy has been repeatedly studied and its frequency has been found to be relatively low compared with, for instance, that associated with the reading of chest radiographs (see “How reliable is smear microscopy?”, page 14, and “How reliable is chest radiography?”, page 51). Several studies have been carried out to compare the results of different readers who independently examined smears prepared from the same specimens. When the readers were asked whether the smear was positive for acid-fast bacilli, the frequency of agreement was 93%. Such a high level of agreement has never been observed among readers of chest radiographs, even in response to such basic questions as: “Is the lung radiograph normal?” and “Is there a cavity present?” (see “How reliable is chest radiography?”, page 51).

Many reader errors would be avoided if microscopists were properly trained and strongly advised to report what they actually see (rather than what they think they are expected to see). Diagnostic bias in favour of sickness – or, in treated patients, in favour of cure – is a known reason for reading error. However, discrepancies in the results of smear microscopy are due far more often to deficiencies in sputum collection and smear preparation than to reader errors.

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7. What are the main consequences of false-positive and false-negative sputum smears?

*T. Frieden*¹

False-positive smears

The main consequences of false-positive sputum smears are (1–3):

False-(over-)diagnosis of tuberculosis

As a result:

- *Patients and their contacts are started on tuberculosis treatment unnecessarily, with possible complications.* Drug interactions can also cause problems if patients and their contacts are receiving other medications.
- *Delay in a correct diagnosis.* Once a positive result is obtained, the patient is started on tuberculosis drugs; further investigations for other diagnoses are generally not conducted. As the response to treatment in tuberculosis is slow, many clinicians wait 1–2 months or more before considering an alternative diagnosis. This delay in establishing the correct diagnosis may lead to increased morbidity and mortality from the actual non-tuberculous condition.
- *Emotional stress.* Many patients suffer emotional stress as a consequence of the diagnosis. In many societies, the diagnosis of tuberculosis still carries significant stigma.
- *Medications will be wasted.* A false-positive sputum test will result in unnecessary administration of antituberculosis drugs.
- *Financial loss.* Free treatment is not available everywhere for all patients. A false-positive diagnosis may therefore be associated with an unnecessary financial burden on the patient.
- *Patients and the community may lose confidence in the tuberculosis control programme.* Health workers should tell patients and the community that patients must continue to take medicines for 6 months, failing which they may become severely ill or even die. However, if a smear was falsely positive and a patient does not in fact have tuberculosis, that patient may discontinue treatment after only a few weeks and feel completely healthy. This may reduce the likelihood that patients who

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actually have tuberculosis will present for care promptly and take medicines as prescribed. The confidence of the community is important for effective programme implementation.

- *Unnecessary evaluation and treatment of children* if contact examination is undertaken.

False information about the progress or outcome of treatment

As a result:

- *Treatment may be continued for longer than necessary*, in the case of false-positive follow-up examinations.
- *Patients may be incorrectly considered to have failed to respond to treatment* and be given re-treatment regimens unnecessarily.

False-negative smears

The main consequences of false-negative sputum smear results are (1–3):

- *Patients with tuberculosis may not be treated, resulting in suffering, spread of tuberculosis and death.* If tuberculosis is not diagnosed and treated, it may become more severe and lead to destruction of the lung parenchyma, with extensive fibrosis. The resulting loss of lung function will be greater than if treatment had been initiated at an earlier stage. The disease may also spread to other people in the community.
- *Patients, physicians and the community may lose confidence in the programme.*
- *Treatment of infectious patients may be inadequate* (Category III instead of Category I, see “What are the diagnostic categories and what is the rationale for these categories?”, page 128) *and of insufficient duration* (in the case of smears taken at the end of the intensive phase). This may result in an increased risk of drug resistance, inadequate treatment, relapse, and spread of tuberculosis. When clinical suspicion of tuberculosis is high, empirical treatment may be started but with fewer drugs than required because the patient is considered to be smear-negative. This may lead to treatment failure and possibly to emergence of drug resistance.
- *Unnecessary investigations.* False-negative sputum smears may lead to lengthy and unwarranted investigations for other conditions.
- *Financial loss.* Delay in the diagnosis of tuberculosis may result in expensive tests being done in the search for other possible conditions.

Laboratory staff must be adequately trained, supported, and supervised so that the preparation, staining, and reading of sputum smears for acid-fast bacilli are carried out correctly and consistently. Quality control of smears is essential (4). Accuracy of results is of particular importance where diagnosis of pulmonary tuberculosis is based mainly on sputum smear examination.

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8. What are the advantages and disadvantages of fluorescence microscopy?

*K. Toman*¹

Fluorescence microscopy for the detection of acid-fast bacilli was introduced in the 1930s. At first, the microscopes had many technical shortcomings: they were difficult to handle and had to be used in dark rooms. Because of these difficulties, fluorescence microscopy was not widely accepted. The microscopes have since been substantially improved, and examination of sputum smears by fluorescence microscopy has become a well-established method in some high-volume laboratories.

The main advantage of fluorescence microscopy is that it uses a low-power (25×) objective. The field seen is thus many times larger than that seen in conventional bright-field microscopy through an oil-immersion objective: in fluorescence microscopy the field is about 0.34 mm², whereas that seen with an oil-immersion objective is only about 0.02 mm². Fluorescence microscopy allows the same area of a smear to be scanned in a much shorter time than can be achieved by conventional microscopy after staining with the Ziehl–Neelsen technique. A microscopist can properly examine at least 100 smears per day by fluorescence microscopy compared with only 30–40 Ziehl–Neelsen-stained smears (1–3).

Since about 15 times as many fields can be scanned by fluorescence microscopy as by conventional microscopy in the same period, there is a higher probability of finding AFB, particularly if a smear contains only a few bacilli. This was confirmed by a large comparative study, which showed that fluorescence microscopy carried out for 1 minute gave more true-positive – and no more false-positive – findings than conventional microscopy for 4 minutes, as judged by culture results (1).

The two techniques have been compared in a number of studies. In one investigation, 175 sputum specimens were examined in parallel (David et al., 1975, unpublished data). Duplicate smears were prepared from each specimen and examined independently by conventional microscopy and by fluorescence microscopy. The results obtained with each technique were recorded for every pair of smears separately and used to construct a correlation table (Table 8). Results that were identical are plotted on the diagonal. If the differences in grading of positive smears were disregarded, 157 of the 175 pairs of smears gave identical results, i.e. there was 90% agreement.

¹ Deceased.

Table 8
Correlation between bright-field microscopy (Ziehl–Neelsen technique) and fluorescence microscopy

Ziehl–Neelsen microscopy	Fluorescence microscopy		Total
	0 or scanty	Positive	
0 or scanty	10	12	116
Positive	6	53	59
Total	110	65	175

In another study comparing both techniques with the culture method (3), 1383 sputum specimens were collected, a pair of smears and one culture being made from each. The smears were examined independently, one by conventional Ziehl–Neelsen microscopy and the other by fluorescence microscopy (Table 9). The main purpose of the study was to assess the efficacy of each technique compared with culture. Another aim was to see whether fluorescence microscopy yielded false-positive results and, if so, how many. This information was essential because it had been suggested that sputum might often contain naturally fluorescent particles that could be confused with AFB (4).

For convenience of comparison, the data from Table 9 are been presented in two separate forms in Table 10. Comparison of the positive yield of fluorescence and Ziehl–Neelsen microscopy with that of culture showed a very slight advantage in favour of fluorescence microscopy. Of the 655 specimens that were positive by culture, 441 (67.7%) were positive by fluorescence microscopy and 433 (66.1%) by conventional microscopy.

There was practically no difference between the two methods as regards false-positive results. Of the 456 specimens positive by fluorescence microscopy, 15 (3.3%) were not confirmed by culture, compared with 14 (3.1%) of 447 specimens positive by conventional microscopy. In other words, 97% of the positive yield of either technique was unequivocally confirmed by culture. Thus, the fears about low specificity of the fluorescence technique seem to have been unwarranted (5). The examinations were carried out by regular laboratory personnel with experience in fluorescence microscopy. The results may thus be regarded as a standard performance for reasonably competent technicians. More recent studies confirm the similar results of using both methods in field conditions. Nevertheless, care must be taken to avoid a small number of inorganic acid-fast objects being mistaken for a scanty positive smear. With

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Table 9
Results of examining 1383 sputum specimens by fluorescence microscopy (FL) and Ziehl–Neelsen microscopy (ZN) and by culture^a

Category	Smear results		Specimens	
	FL	ZN	No.	%
1 Smear +) Culture +)	+	+	405	33.9
	+	–	36	
	–	+	28	
2 Smear +) Culture –)	+	+	11	1.2
	+	–	4	
	–	+	3	
3 Smear –) Culture +)	–	–	186	13.4
4 Smear –) Culture –)	–	–	681	51.5
Contaminated cultures	–	–	29	
Total			1383	100.0

^a Source: reference 3.

Table 10
Comparison of fluorescence microscopy with culture and Ziehl–Neelsen microscopy with culture

	Fluorescence microscopy		Total		Ziehl–Neelsen microscopy		Total
	+	–			+	–	
+	441	214	655	+	433	222	655
Culture							
–	15	713	728	–	14	714	728
Total	456	927	1383	Total	447	936	1383

regard to possible scanty false-positive results by fluorescence microscopy, the adage, "All that glistens is not AFB" should be remembered.

The disadvantages of fluorescence microscopy are the relatively high costs of a microscopy unit and of its maintenance. Nevertheless, in central or other large laboratories where the workload exceeds that of three technicians working with three conventional microscopes (e.g. more than 100–150 slides/day), it may be cheaper to use one fluorescence microscope instead. That calculation applies to places where the salaries of technicians are low, e.g. in developing countries (6). In countries where salaries are higher, fluorescence microscopy is generally less expensive than conventional microscopy even at lower volumes, because it requires fewer costly personnel (7, 8).

A further disadvantage of fluorescence microscopy is that the handling and maintenance of the optical equipment require advanced technical skill. The fluorescence microscope is also less robust than conventional instruments. Component parts, particularly bulbs, have to be replaced from time to time, and may be expensive and difficult to procure; repairs are occasionally necessary. In addition, a continuous supply of standard electrical power with minimal voltage fluctuations is needed. These requirements are often difficult to meet in developing countries. It should be noted that reference laboratories that adopt fluorescence microscopy must continue to use light microscopy for quality control and for training of field staff.

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9. What is the role of mycobacterial culture in diagnosis and case definition?¹

A. van Deun²

Role of mycobacterial culture in the diagnosis of tuberculosis

The probability of finding acid-fast bacilli (AFB) in sputum specimens by smear microscopy is directly related to the concentration of bacilli in the sputum (see “How many bacilli are present in a sputum specimen found positive by smear microscopy?”, page 11, and “How reliable is smear microscopy?”, page 14). At concentrations below 1000 organisms per ml, the chance of observing bacilli in a smear becomes less than 10%. In comparison, mycobacterial culture can detect far lower numbers of AFB, the detection limit being around 100 organisms per ml. Moreover, culture makes it possible to identify the mycobacterial species on the basis of biochemical and other properties. Smear microscopy cannot reliably differentiate between the various pathogenic and non-pathogenic mycobacteria, which are all acid-fast and morphologically alike. It therefore seems that, for the diagnosis of tuberculosis, both the sensitivity and the specificity of culture methods are far better than those of smear microscopy.

In practice, however, the diagnostic effectiveness of any method will also be influenced by its sensitivity to technical deficiencies and by the circumstances in which it is used. In the case of AFB smear microscopy, technical errors almost never affect the extremely high specificity (see also “How reliable is smear microscopy?”, page 14). Because of its higher sensitivity, *Mycobacterium tuberculosis* culture is more susceptible to reduced specificity as a result of contamination: various manipulations may result in transfer of bacteria from positive to negative samples. To investigate the magnitude of this problem in the East African collaborating laboratories of the British Medical Research Council, positive sputum specimens marked with a rifampicin-monoresistant strain were mixed with negative samples. This exceptional strain could later be traced back to nearly 1% of the negative specimens (1). Even at a true positivity rate of about 25% within these series, this meant that 1.6–4.7% of culture isolates in fact constituted false-positives. More recently, using DNA fingerprinting

¹ Based on the chapter by K. Toman in the first edition.

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techniques, similar percentages of cross-contamination have been found repeatedly in routine laboratories in countries where the prevalence of tuberculosis is low (2, 3).

In countries with a high prevalence of tuberculosis, the specificity of smear microscopy may thus be superior to that of culture. This may be true even for the diagnosis of tuberculosis (4), since AFB demonstrated in direct sputum smears would then almost invariably represent *M. tuberculosis*, even in areas with a high burden of HIV (5). By contrast, in countries with a low prevalence of tuberculosis, culture (or alternative techniques of species identification) will often be indispensable to the differentiation of tuberculosis from other mycobacterial diseases.

Furthermore, health services in high-prevalence countries are often inaccessible because of geographical, financial, or cultural factors, and patients frequently present at an advanced, cavitary stage of the disease. The concentration of bacilli in the sputum is determined largely by the type of tuberculous lesion from which the bacilli originate. Thus, a cavity about 2 cm in diameter (opening into a bronchus) may contain some 100 million tubercle bacilli, whereas a non-cavitated nodular lesion of the same size may contain only 100–1000 bacilli (6). Sputum from patients with tuberculous lung cavities that contain softened necrotic particles with enormous numbers of bacilli will almost invariably be found positive by direct smear microscopy. In contrast, sputum from patients with nodular, encapsulated lesions discharging only small amounts of bacilli will usually be negative by smear microscopy. This pathology-related aspect of susceptibility was clearly shown in a study by Kim et al. (7) that compared radiographic severity and extent of culture-positive disease with microscopy results in concentrated sputa (Figure 1).

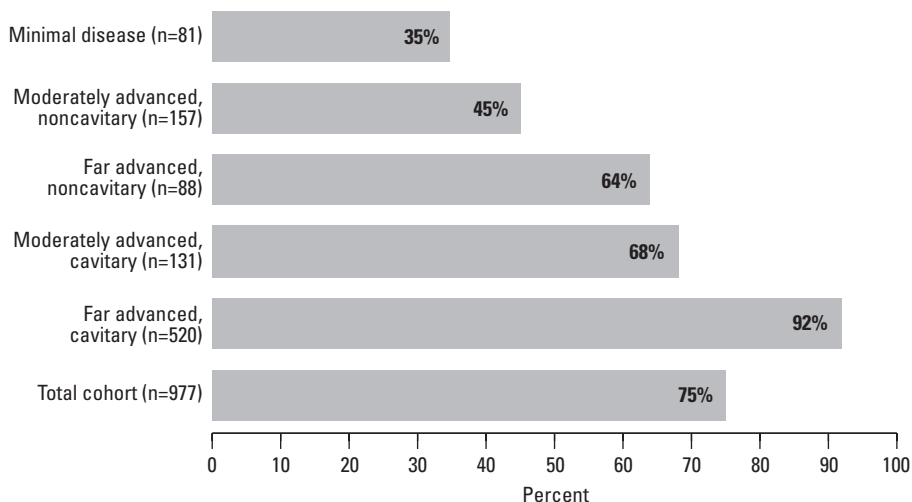
With this background, it is also easy to understand that the difference in sensitivity between culture and microscopic detection will be greater in active case-detection or in surveys. Substantially more cases will then be encountered with less severe or even subclinical disease, and a smaller proportion of cases will have reached a cavitary stage with high numbers of bacilli. This was illustrated by a comparison of the yield from microscopy versus culture in different surveys and studies conducted by the National Tuberculosis Institute in Bangalore, India. While microscopy could detect only 40–50% of culture-positive cases found in the surveys, its yield rose to about 85% in persons self-reporting with chest symptoms (8).

Provided that careful techniques are used, the diagnostic yield from smear microscopy can still be high in the context of HIV (9, 10) (see also “How does the diagnosis of tuberculosis in persons infected with HIV differ from diagnosis in persons not infected with HIV?”, page 80). The relative sensitivity of culture and microscopy is illustrated by Table 11 below from a publication by Urbanczik (11). Since then, even higher rates (more than 80%) have been reported from high-prevalence areas, including those with a serious HIV burden (5, 10).

It thus appears that the yield of microscopy compared with culture is highly variable in practice. Some of the observed variation can be explained by differences

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Figure 1
Percentage of smear-positive cases out of all culture-positive pulmonary tuberculosis patients by severity of disease on chest radiography^a



^a Source: reference 7.

Table 11
Percentage smear-positive out of all culture-positive pulmonary tuberculosis^a

Country/area	Year	Percentage smear- and culture-positive
USA	1976	62
USA	1975	22
USA	1976	43
Africa/Europe	1980	53 (Ziehl-Neelsen)
Asia/USA	1980	63 (fluorescence)
USA	1975	24
United Kingdom	1992	53
Germany	(no date)	54
USA	1977	50
USA	1980	25
Germany	(no date)	37

^a Modified from reference 11.

between populations (high- versus low-prevalence countries, early or late case presentation) and details of the techniques used (e.g. fluorescence microscopy, concentration techniques). Some, however, must be due to deficiencies in the execution of the tests.

Despite the higher sensitivity of culture, use of the technique may not be particularly rewarding for the examination of persons presenting spontaneously with chest symptoms. In high-prevalence countries, with or without HIV being present, and given correct use of both methods, the gain by culture over microscopy is estimated to be about 25% (12). In low-prevalence countries, this gain will be greater, possibly doubling the proportion of patients with positive bacteriological findings. Moreover, culture has the added advantage of allowing identification of the mycobacterial species, which is not possible with microscopy.

Thus, from the bacteriological point of view, two main categories of patient may be distinguished: one much more infectious, discharging large numbers of tubercle bacilli in almost every sputum specimen and easily detectable by microscopy, and the other much less infectious, discharging smaller numbers of bacilli, usually not found except by culture. As mentioned earlier, patients in the latter category may discharge bacilli only intermittently (see "What is the additional yield from repeated sputum examinations by smear microscopy and culture?", page 46). Obviously, these two categories also differ significantly in clinical and epidemiological respects.

Sputum status and clinical prognosis

The prognosis for patients with pulmonary lesions discharging small numbers of bacilli, demonstrable only by culture, is generally more favourable prognosis than that for smear-positive patients. In southern India, where an epidemiological survey had been repeated at intervals, the fate of newly discovered cases was analysed (13, 14). Of patients who had been smear-negative (two specimens) but positive by culture at the time of detection of their disease, more than half were classified as cured (i.e. negative by both smear and culture) within 18 months and about two-thirds within 3 years. Moreover, the excess death rate was about one-third of that for smear-positive cases. Thus, even under the living conditions of a very poor rural population, and without treatment, the prognosis for smear-negative, culture-positive patients was relatively favourable.

Though smear-negative cases were known to have a lower mortality, they were thought to be at an early stage of the disease and it was assumed that they would deteriorate further and become smear-positive later on. To prevent this, it was often considered important to detect patients "early", i.e. at a stage where the extent of the disease is minimal and the lesion(s) are likely to contain a small number of bacilli demonstrable only by culture. It has also been assumed that these patients rarely have symptoms and thus may be detected best by indiscriminate mass radiography. Surprisingly, this hypothesis has not withstood the test of time.

In a carefully conducted longitudinal study (see “How does pulmonary tuberculosis develop and how can it be detected at an early stage?”, page 66), the population of a district in the then Czechoslovakia underwent radiological and bacteriological examinations at intervals of 2–3 years (15). Coverage of the eligible population was almost complete (95%). With each round, appreciable numbers of new patients were detected who had small radiographic lesions and were positive by culture but negative by smear microscopy (specimens collected on 3 consecutive days). All these patients were immediately put on treatment, which was successful. According to the hypothesis, these patients were prevented from deteriorating and developing advanced, smear-positive tuberculosis. It was therefore expected that the frequency of new smear-positive cases would decline rapidly as a result. However, the intensive case detection and treatment measures had surprisingly little effect: despite extensive efforts, at and between examination rounds, a large proportion of newly detected cases were already at an advanced stage and were smear-positive.

These and other studies have thus shown that the development of new smear-positive tuberculosis does not necessarily go through an early, smear-negative stage (see “How does pulmonary tuberculosis develop and how can it be detected at an early stage?”, page 66). The prognosis for patients with smear-negative but culture-positive lesions has proved to be far better than was previously assumed, with most such lesions either healing or remaining unchanged. Only a few patients deteriorate, and thus only a few smear-positive cases would be prevented by the use of additional, more sensitive detection methods such as mycobacterial culture.

Sputum status and infectivity

From the epidemiological point of view, as well, the difference between these two types of case is striking. Patients who are definitely negative by smear are substantially less infectious than are smear-positive patients. This is not surprising, in view of the enormous difference in the numbers of bacilli discharged by the two categories of patient. The risk of exposure to smear-positive sources of infection is aggravated because such persons usually cough more frequently and violently (16). For household contacts of smear-negative, culture-positive patients, for instance, the excess risk of becoming infected is only a small fraction of that for household contacts of smear-positive patients. Excess risk is the risk in addition to the basic risk of exposure to the immediate, extra-domiciliary, neighbourhood. The basic risk is naturally higher in crowded or slum conditions than the average risk for the total population – often wrongly used for comparison. Moreover, the risk for household contacts of contracting the disease from culture-positive, smear-negative patients is only about 10–20% of that for contacts of smear-positive patients (17, 18) (see “What is the role of case detection by periodic mass radiographic examination in tuberculosis control?”, page 72). These findings have been confirmed by DNA fingerprinting studies on the relatedness of strains of *M. tuberculosis* isolated in San Francisco, USA, from 1991 to 1996: only 17%

of the disease transmission could be attributed to smear-negative, culture-positive index patients (19).

The detection of patients with lesions that harbour and discharge small numbers of bacilli thus seems to have a relatively low priority in tuberculosis control. If identification of such patients is attempted in a tuberculosis programme, it should never be at the expense of the top priority of case detection, i.e. the identification of sources of infection. Taking into account all the rates mentioned so far, it is certain that more than 90% of the sources of infection in high-prevalence countries can be detected by well-executed smear microscopy.

There are other reasons why mycobacterial culture is less used in tuberculosis diagnosis. In high-prevalence countries, culture facilities are scarce and difficult to set up because of financial and technical constraints. Mycobacterial culture using conventional media (egg-based, i.e. Löwenstein–Jensen medium, or agar-based, i.e. Middlebrook medium) is 5–10 times more costly per sample than smear microscopy. The necessary equipment is more difficult to obtain and trained personnel more difficult to find. Even in centres where such culture facilities are available, the method is used mainly for confirmation of diagnosis in cases already being treated for tuberculosis. This is because most cultures become positive about 3 weeks after inoculation, so that the results of conventional mycobacterial culture are available only after a delay of at least a month. Clinicians will not wait for the results, particularly since a small proportion of cases yield negative cultures and are diagnosed only on radiography. Radiographic diagnosis as part of a systematic diagnostic algorithm will thus be automatically preferred to culture (see “What are the relative merits of chest radiography and sputum examination (smear microscopy and culture) in case detection among new outpatients with prolonged chest symptoms?”, page 61). Using modern liquid media and more sensitive growth detection systems, positive culture results can be available earlier – within 1–2 weeks. However, the commercial systems needed for this are costly to install and operate, and demand a high level of technical expertise. Moreover, diagnostic evaluation based on a standard algorithm will generally result in treatment being initiated at least as quickly, and much more cheaply, than culture based on these systems.

For all these reasons, the role of mycobacterial culture in the diagnosis of tuberculosis becomes more important only with declining prevalence of the disease. As prevalence falls, clinicians will be less likely to suspect, and less expert in recognizing, the disease, so that even a late culture result will be useful. Cases will present with less severe disease, with consequently lower proportions of smear-positive tuberculosis. Other less pathogenic mycobacteria will become relatively more frequent etiological agents, making species identification of the various AFB essential. Finally, a decline of tuberculosis will generally be accompanied by economic improvement, making installation of equipment and optimal use of culture more feasible.

As long as tuberculosis remains highly prevalent, the role of mycobacterial culture will be secondary to that of careful smear microscopy and radiographic/clinical

diagnosis. If available, culture should be used for extrapulmonary tuberculosis easily accessible to sampling, e.g. glandular tuberculosis. In HIV-positive patients particularly, this would greatly reduce diagnostic and treatment error. Culture also remains essential for susceptibility testing for the surveillance of drug resistance.

Role of mycobacterial culture in tuberculosis case classification

Mycobacterial culture is useful for definitive confirmation of tuberculosis. However, under programme conditions, the role of culture can be largely taken over by microscopy. The longitudinal surveys in southern India (15), which provided insight into differential survival, were also analysed in this respect. Culture and smear microscopy had been done for all persons considered probably to be suffering from active tuberculosis on radiographic screening, with follow-up of the evolution in the absence of treatment. Provided that a threshold for positivity of more than three AFB per smear was respected, only 10% of smear-positive cases failed to yield growth of *M. tuberculosis* in culture. By contrast, almost two out of every three radiological cases could not be confirmed by culture, nor was there other evidence of progressive tuberculosis. Later studies (20) have confirmed the reliability of smear microscopy as a proxy for culture in the classification of tuberculosis cases, with only 3–6% of smear-positives having negative cultures. Many of these apparent false-positives of microscopy may be due to treatment, since rechecking of slides can often confirm the presence of (non-viable) AFB.

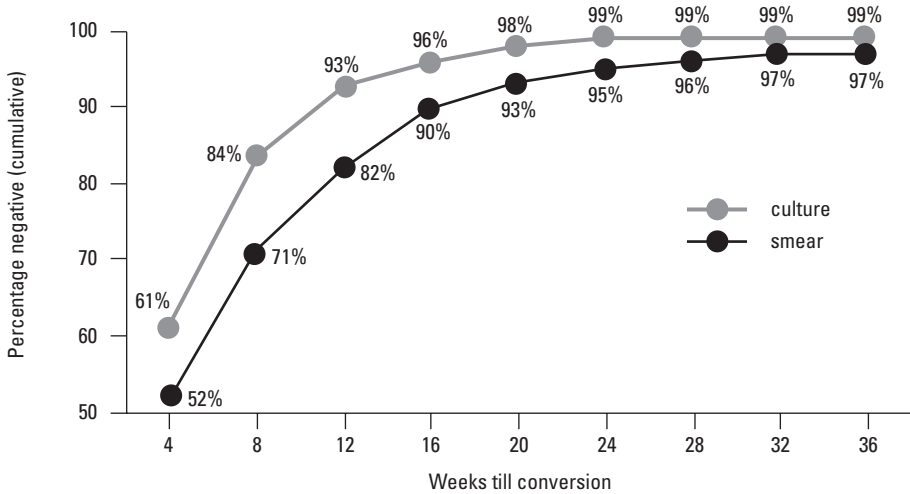
The definitions of “sputum smear-positive” and “sputum smear-negative pulmonary tuberculosis case” take into account the limited sensitivity of smear microscopy. At the same time they emphasize the primary importance of smear-positive pulmonary tuberculosis cases for control of the disease.

Culture might be more relevant for the definition of cure, failure, and relapse. Several studies, for example that by Al-Moamary and colleagues (21), have documented the delayed conversion of smear from positive to negative compared with culture (Figure 2).

In the sputum of some patients, non-viable bacteria remain microscopically visible even after 5 months or more of treatment. In a study by Rieder (22), only 2 out of 8 cases that were smear-positive at 5 months or later needed re-treatment. Culture allows a more accurate classification of such cases, but it is not a practical solution in most areas: for programme purposes, failure is defined as the presence of bacilli after 5 months or more of treatment. Another cause of sputum smear-positive, culture-negative results is laboratory error.

On the other hand, without culture, some patients in whom treatment fails will go unrecognized, especially if microscopy is not performed well. These patients may present as early “relapse”. Microscopy-based classification of both failure and relapse is thus less reliable. However, with the scarcity and complexity of facilities and the delay in culture results, the definitions of cure, failure, and relapse are

Figure 2
Conversion in smear and culture of initially smear-positive pulmonary tuberculosis patients^a



^a Source: reference 21.

based primarily on smear examination, with culture as an option where this is available.

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10. What is the probability of obtaining a negative culture from a sputum specimen found positive by smear microscopy?

*K. Toman*¹

In a study of the efficacy of bacteriological measures under the conditions of the Singapore tuberculosis control programme (1), 1162 new patients with clinical and radiological signs suggesting tuberculosis were examined as follows.

Two sputum specimens were collected from each patient – one on each of two consecutive days – in the presence of a trained supervisor. All specimens were examined independently by direct smear microscopy in one laboratory and by culture in another laboratory (one smear and one culture per specimen). Of the 1162 patients, 500 had a positive smear from one or both specimens, as shown below.

	Number of new patients smear-positive for acid-fast bacilli
Yield from first specimen	428
Additional yield from second specimen	72
Total	500

The results of two culture examinations of the sputum of these smear-positive patients are given in Table 12. In 17 of 500 patients, i.e. less than 4%, the positive smear results were not confirmed by two culture examinations. Assuming that the contaminated cultures were all negative, the proportion of unconfirmed results would amount to 6% at the most. A further analysis (not tabulated) showed that, of the 115 patients found positive in only one of two smears, 101 (almost 90%) were confirmed by culture to be excreting tubercle bacilli.

The authors concluded from these results that, when tubercle bacilli were identified by smear examination, culture examination of two specimens confirmed the smear result in all but a very small proportion of cases; hence, culture confirmation of a positive result based on two smear examinations did not appear to be necessary. This is particularly true in populations with a high prevalence of tuberculosis, where

¹ Deceased.

Table 12
Results of culture examinations on two consecutive sputum specimens from 500 new patients smear-positive for acid-fast bacilli

	No. of patients	%
Total examined	500	100
Confirmed by first culture	399	80
Confirmed by second culture (additional)	73	14
Contaminated (both cultures)	11	2
Not confirmed by either culture	17	4

patients seek medical attention only because of haemoptysis or prolonged chest symptoms such as a productive cough.

A negative culture result with a specimen containing tubercle bacilli may be due to various causes. In patients receiving treatment, the organisms may have lost their ability to grow on culture media and be practically dead. Patients being treated with a rifampicin-containing regimen often become culture-negative by about the third week of treatment, although they may still be sputum smear-positive: bacilli are dead or non-viable. In patients who have not had treatment, sputum specimens may have been exposed to sunlight or heat, stored too long, dried out, or contaminated. Excessive decontamination procedures before inoculation, over-heating during centrifugation, inadequate culture media, and deficient incubation may also result in a negative culture. In a few instances, positive smears may be caused by non-tuberculous mycobacteria.

The probability of obtaining a positive culture in patients with both sputum specimens smear-negative is a quite different problem, dealt with under the heading "What is the role of mycobacterial culture in diagnosis and case definition?" (page 35).

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11. What is the additional yield from repeated sputum examinations by smear microscopy and culture?

*A. Harries*¹

Studies were carried out at the National Tuberculosis Institute of India (1, 2) to determine the additional case yield when eight sputum specimens from each individual with suspected pulmonary tuberculosis were examined by both smear microscopy and culture (see also “What are the relative merits of chest radiography and sputum examination (smear microscopy and culture) in case detection among new outpatients with prolonged chest symptoms?”, page 61).

For each of 194 individuals who had abnormal radiographic lung shadows and complained of prolonged chest symptoms suggestive of tuberculosis, eight successive sputum specimens (four collected on the spot and four produced overnight and collected by a home visitor) were examined concurrently by smear microscopy (Ziehl–Neelsen method) and culture. The number of specimens thus examined was 1552; each was examined independently, the laboratory technician having no knowledge of the persons examined or of previous results. Tubercle bacilli were found in the sputum of 75 patients (Table 13).

Table 14 shows the yield of new cases resulting from examination of the first and subsequent specimens, in chronological order. In successive examinations – whether by smear microscopy or by culture – most new positive results are clearly obtained from the first and second specimens. The upper half of the table shows that 45 (85%) of all smear-positive patients were already positive by examination of the first two specimens; for the smear-positive cases confirmed by culture, 41 (89%) were positive on the basis of the first two examinations. Thus, a second culture increased culture sensitivity from 63% to 84%. Accordingly, the optimal number of cultures is two, or at the most three.

Another important finding of this investigation (Table 14) was that the first two smears detected about the same number of new cases (45) as the first culture examination (43). It may therefore be concluded that, in new, untreated patients with prolonged chest symptoms and abnormal lung radiograph shadows, two consecutive smear examinations (e.g. of on-the-spot and overnight sputum) are practically

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Table 13
Results of concurrent examinations of eight sputum specimens by smear microscopy and culture

(For each of 194 patients, four specimens were collected on the spot and four in the early morning.)

Specimens examined	1552
Patients examined	194
Patients negative (all smears and cultures)	119
Patients positive:	
at least one smear and one culture	46
at least one culture (all smears negative)	22
at least one smear (all cultures negative)	7 ^a
	} 75

^a Two of these patients had smears with three or fewer acid-fast bacilli.

Table 14
Yield in cases from concurrent smear (S) and culture (C) examinations of eight consecutive sputum specimens from each of 194 persons with lung radiographic shadows and prolonged chest symptoms suggesting tuberculosis

Bacteriological category	No. of cases	Number of cases according to serial number of specimen yielding first positive result								
		I	II	III	IV	V	VI	VII	VIII	
S+	46	34	7	1	1	–	–	1	2	
C+		41 (89%)								
Smear-positive	S+	7	2	2	–	–	–	1	1	1
	C–									
	Total	53	36	9	1	1	–	1	2	3
			45 (85%)							
	C+	46	34	7	1	1	–	–	1	2
	S+									
Culture-positive	C+	22	9	7	1	1	1	1	2	–
	S–									
	Total	68	43	14	2	2	1	1	3	2

equivalent to one culture examination. This inference concords with the results of other studies, such as that undertaken in new patients attending the Tuberculosis Research Centre, Chennai, India (formerly the Tuberculosis Chemotherapy Centre, Madras) (3), and another study on bacteriological case-detection procedures in patients attending health services in Singapore (4). In the latter study, covering 1162 new patients with radiographic signs and chest symptoms suggestive of tuberculosis, culture of the first sputum specimen revealed 535 cases, whereas two consecutive smears detected 500 cases (see "What is the probability of obtaining a negative culture from a sputum specimen found positive by smear microscopy?", page 44).

All the above-mentioned findings confirm Mitchison's observations (5) that: "... smear examination, especially of several specimens from each patient, is almost as efficient as culture examinations in clinics in developing countries." This may apply also to other high-prevalence situations or to preselected groups of patients who have been prompted by symptoms (such as prolonged cough, purulent sputum, and haemoptysis) to attend a health centre (6).

Another significant observation in the study was that the 46 patients who were found positive by smear and culture were discharging tubercle bacilli practically every day (of a total of 368 specimens from these 46 patients, 347 (94%) were culture-positive). In contrast, out of 176 specimens from patients negative by smear microscopy and positive only by culture, only 62 (35.2%) were positive on culture. This latter category of patients therefore discharges bacilli only about every third day or in only every third specimen (see Table 11 in "What is the role of mycobacterial culture in diagnosis and case definition?", page 35). This confirms that patients positive only by culture and negative by smear microscopy have significantly less epidemiological impact than those who are positive by smear (and culture).

A similar study was carried out at the same institute in connection with an epidemiological survey of a district of southern India (2). Eight consecutive sputum specimens were collected from each of 1652 persons with an abnormal chest radiograph and examined as in the study previously reported. The results were comparable. In 86.7% of those positive by smear microscopy and culture, the first smear was positive, with the second smear adding another 10% of positive results. In those who were positive only by culture, the first specimen yielded only 32% of positive results, and the second yielded 18%. It was also observed in this study that patients discharging large numbers of bacilli provided positive specimens almost every time, whereas those who were culture-positive but smear-negative frequently produced specimens containing no bacilli (see "What is the role of mycobacterial culture in diagnosis and case definition?", page 35).

In areas of sub-Saharan Africa with a high prevalence of HIV, evidence suggests that two sputum smears could also serve as the basis for evaluation of chronic cough. In the United Republic of Tanzania (7), the routine results of direct examination of sputum smears for acid-fast bacilli from 61 580 patients with suspected tuberculosis were analysed. The average proportion of smear-positive cases found was 18.9%.

Among patients in whom a complete set of three sputum smears was examined, the incremental yield of smear-positive cases was 83.4% with the first specimen, 12.2% with the second specimen, and 4.4% with the third specimen. In a study in Malawi (8) of 280 persons with chronic cough, weight loss, and no improvement after a course of antibiotics, 71 patients were sputum smear-positive. Among patients with smear-positive tuberculosis, the diagnosis was made from the first specimen in 83% of cases, from the second specimen in 13%, and from the third specimen in 4%.

In one district in Malawi, two- and three-smear strategies were compared for 6 months (9). In both, 16% of patients with suspected tuberculosis were smear-positive. The clinical pattern of tuberculosis, especially with regard to smear-positive pulmonary tuberculosis cases, was similar with the different strategies. The strategies with two and three sputa were compared in an area of rural Africa with high HIV prevalence (10), with fluorescence microscopy and confirmation of the positive smears with Ziehl-Neelsen staining. Of the cases detected with three smears, 97% would have been detected with the first two.

Three smears are preferable. The vast majority of patients with positive smears will have two or three positive smears, whereas in patients with a single positive smear the result may be a false-positive because of mislabelling or technical error (see “What are the main causes of false-positive and false-negative sputum smears?”, page 23). Even in otherwise well-functioning microbiology laboratories, 1–4% of positive cultures may be false-positives (11, 12). This supports use of the algorithm recommended by WHO, in which further evaluation is required if patients have only a single positive smear. In addition, three smears cause no greater inconvenience to the patient than two if they are done in 2 days (spot – early morning – spot). However, in areas where human and financial resources are significantly limited, a two-sputum smear strategy has been successfully employed.

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12. How reliable is chest radiography?¹

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The introduction of radiography as a diagnostic tool was a landmark in our knowledge of the natural history of tuberculosis and its diagnosis. It is therefore no wonder that the enthusiasm with which radiography was received and applied sometimes caused the method to be overrated. It is still widely believed that pulmonary tuberculosis can be diagnosed by chest radiography alone. However, practical experience and many studies have shown that no radiographic pattern is diagnostic of tuberculosis. Many diseases of the lung show a similar radiographic appearance and can easily mimic tuberculosis. Similarly, the lesions of pulmonary tuberculosis can take almost any form on a radiographic picture (1).

Chest radiography can help to localize abnormalities in the lung, but further examination is required in order to establish the tuberculous etiology of an abnormality, and only bacteriology can provide proof.

Observer error

Many widely used clinical tests and laboratory procedures that are regarded as precise and objective are in fact subject to varying degrees of observer error. Examples of such tests include blood-pressure measurement, electrocardiography, manual blood cell counts, endoscopies, visual colorimetric tests, and chest radiography. The usefulness of chest radiography is determined largely by the reader's ability to detect abnormal opacities and interpret them correctly. This implies not missing or under-reading radiographic opacities and, conversely, not over-reading normal opacities as abnormalities. This ability may vary not only from one reader to another (inter-observer variation), but also between viewings of the same film by a single reader (intra-observer variation).

Observer error in interpretation of chest radiographs was studied several decades ago when antituberculosis campaigns were started in many developed countries. Most of

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the early studies were designed or conducted by Yerushalmy, a biostatistician, to explore the efficacy of various radiographic and photofluorographic techniques and equipment.

Over- and under-reading

One trial, designed to examine the effects of the size of film on the results of chest radiography, suggested that this variable was far less important than the degree of observer variation (2). Each of five experts missed (under-read) approximately 25% of the "positive" films in each series (Table 15). When the same films were read again after about 3 months, the experts changed their mind in about one-fifth of the cases they had previously classified as "positive" (an intra-individual inconsistency of 20%).

Additional studies confirmed that 26–43% of films might be under-read (3, 7, 8). A Danish group (5, 9) consisting of three experienced readers examined 5000 unselected small films independently (Table 15). On average, under-reading occurred in 32% of cases and over-reading in 2%. These observations were later confirmed in the United Kingdom (10).

In a large study in the USA (4, 6) on the usefulness of periodic chest radiograph screening, photofluorograms were taken of each of 15000 first-year university students (Table 15). The films were read by a panel of 50 readers composed of equal numbers of radiologists and chest specialists. According to a randomization scheme, each reader provided 3000 readings, thus ensuring 10 independent readings of each film. Students whose films were interpreted as positive by one or more readers were examined by bacteriology, tuberculin test, and tomography, and followed for the duration of their stay at the university. Ultimately, 249 films were classified as

Table 15

Observer error: under-reading and over-reading of radiographs (mostly unselected survey radiographs)

Study (reference)	Under-reading (%)	Over-reading (%)
1. Five expert readers (2)	25	–
2. Readers with varying experience (3)	27	1.7
3. Mass radiography (4)	32	1.7
4. Danish Tuberculosis Index, mass radiography (5)	32	1.6
5. Reader panel (mass radiography of 15000 students, 10 readings per film) (6)		
(a) all 50 readers	39	1.2
(b) the five "best" readers selected from a panel of radiologists and chest specialists		
Group A	21	0.5
Group B	26	0.3

Table 16
Observer error: under-reading and over-reading of chest radiographs^{a,b}

Experience	Number of readers	Under-reading (%)	Over-reading (%)
(a) 1–4 years ^c	37	28.0	18.0
5–9 years	37	19.2	19.0
>10 years	88	17.6	17.0
<i>or</i>			
(b) 1–500 films annually	43	22.4	17.5
5 000–20 000 films annually	48	24.0	18.0
>20 000 films annually	41	15.2	15.5
Average of all readers		21.8	19.5

^a Using a 70 mm mirror reflex camera

^b Source: reference 11.

^c Results from physicians who had practised reading for less than 1 year or had read fewer than 1000 films annually were excluded from analyses.

“definitely positive” by a small group of umpire readers who had access to all the necessary information. The level of under-reading by the whole panel of 50 readers was, on average, 39% of the 249 “definitely positive” films. Conversely, 1.2% (156) of the films were over-read. When only the results of the 10 “best” readers (five radiologists and five chest specialists) were considered, the rates of under-reading and over-reading were appreciably lower, but still unsatisfactorily high (Table 15).

Influence of experience on chest radiograph reading results

The Research Institute of Tuberculosis, Tokyo, examined the extent of over-reading and under-reading by 192 physicians participating in the Japanese National Tuberculosis Case-finding Programme (11). Special attention was paid to the effect of experience in radiograph reading on the degree of reader variation (Table 16).

Radiographs from 50 persons whose health status was well known to the Institute were selected for independent reading; 25 of them had confirmed tuberculosis or other chest diseases, 5 had healed tuberculosis lesions, and 20 no abnormalities. The chosen readers’ experience of reading films varied from less than 1 year to more than 10 years, and they read from 1000 to 20 000 or more films annually (Table 16). They were asked only to decide whether or not further examination was indicated. Failure to request further examination of a person with an abnormality was recorded as under-reading and request for further examination of a person with a normal radiograph was considered as over-reading.

Table 17
Disagreement between readings of chest films of 900 patients^a

Readers	Inter-individual disagreement (%)	Intra-individual disagreement (%)
(a) Two groups of experts (Three radiologists and three chest specialists)		
Group A	29	19
Group B	27	24
(b) Two expert readers (reading the same material)	30	21

^a Source: reference 6.

The average rate of under-reading was 21.8%, and that of over-reading 19.5%. The rates of under-reading among readers who had more than 10 years' experience or who had been reading more than 20 000 films a year were lower about 6–8% than those among the other readers. However, there was not a single reader who did not make at least two misreadings. The investigators estimated that, in the mass radiographic examinations carried out in Japan, probably about one-fifth of cases with active tuberculosis were being missed.

Disagreement between readings of chest radiographs for follow-up

Disagreement among observers occurs not only when radiographs are read for the purposes of case detection and diagnosis, but also when serial films of cases already diagnosed are compared for follow-up. In one study (6), two films (measuring 35.6 × 43.0 cm) taken from each patient at five different times (9000 pairs of films in all) were read. Readers were asked to report whether the second film showed evidence of improvement or deterioration, or no change. The results (Table 17) differed little whether the films were read by two groups composed of three radiologists and three chest specialists respectively, or by two expert readers only. The level of disagreement between readers was 27–30%, and in 19–24% of cases individual readers were likely to disagree with their own earlier reading.

IUAT international study on chest radiography classification

The International Union Against Tuberculosis (IUAT) organized one of the most important comparative studies of the reading and interpretation of chest films. The main goal of the study was to develop a uniform nomenclature and interpretation of radiographic findings that could serve as a basis for an international classification of chest radiographs (12, 13).

A sample of 1100 films was chosen from among several hundred thousand taken during one of the mass radiography surveys of the adult population of Norway. The sample included 200 films from patients with infectious tuberculosis, 400 from

patients with previously active tuberculosis, 100 from persons with minimal findings not requiring referral or follow-up, 300 from persons without abnormal findings, and 100 from patients with verified non-tuberculous lung disease. The films were mounted together in seven film rolls, and 10 copies of each roll were made.

Films were read by 90 experienced physicians (radiologists and chest physicians), 80 of whom were from nine countries where mass radiographic examinations had been carried out for many years: Czechoslovakia, Denmark, Finland, France, Norway, Sweden, the United Kingdom, the United States of America, and Yugoslavia.¹ The remaining 10 readers were selected from WHO project staff.

The study was designed primarily to measure the extent of agreement or disagreement between readers, not the observer error resulting in under-reading or over-reading (14). A set of questions, prepared in advance, was answered by each reader independently. Most of the questions required a "yes" or "no" answer, e.g. "Is there an abnormality in the lung?"; "Is there a cavity present?"; "Does the patient need clinical attention?"

The material was evaluated according to a special statistical procedure (15) by which a series of values was obtained for each question. These values were used to construct a curve that characterized the level of disagreement between readers for a given question (Figure 3). The extent of disagreement was expressed as an index with a value from 0 to 100-0 meaning no disagreement and 100 meaning complete disagreement. The nearer the curve is to the zero point of the two axes, the lower the disagreement; the flatter the curve, or the farther it is removed from the axes, the higher the disagreement. The method showed that there was less disagreement on question 1 than on question 3.

Comparison of reader disagreement in chest radiography and smear microscopy

A similar study was undertaken by IUAT to measure the extent of disagreement between microscopists reading sputum smears for acid-fast bacilli (J. Nyboe, unpublished data, 1971). A series of 250 sputum smears from patients were examined independently in 10 laboratories by experienced laboratory technicians. Figure 4 illustrates the curves of disagreement for three criteria of positivity. The extent of disagreement was lowest (index 10) when the criterion for a positive result was the demonstration of at least eight AFB; it was only slightly higher (index 12) when three AFB were required as the minimum. The extent of disagreement was highest (index 18) between readers when one AFB was accepted as sufficient evidence of positivity. However, even the highest level of disagreement between microscopists was substantially lower than the lowest level of disagreement between readers of chest radiographs.

Curve 1 in Figure 4 illustrates the lowest level of disagreement recorded (index 28)

¹ Country names given are those that were valid at the time of the study.

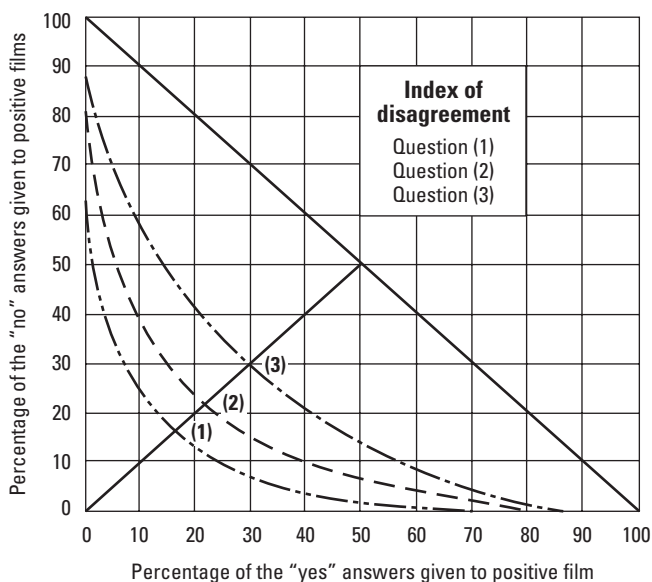
Figure 3

Disagreement between readers in the IUAT study on radiography classification^a

The index of disagreement was based on the following questions:

1. Is there any abnormality in respiratory organs?
2. Is there any abnormality in lymph nodes?
3. Is there any calcification in lymph nodes?

The index is calculated as the sum of percentages of discordant answers at the point on the curve where they are equal. i.e. where the curve is intersected by the oblique line starting from the zero corner.



^a Source: reference 14.

between radiograph readers, i.e. in reply to the question: "Is a cavity present?". Disagreement on the question: "Is the smear positive for acid-fast bacilli?" was substantially lower, whatever the limit chosen – even a single AFB. The investigators concluded that there was consistently better agreement among sputum smear readers, no matter what criterion was used for a positive smear, than among radiograph readers (see also "How reliable is smear microscopy?", page 14).

Levels of disagreement on the interpretation of chest radiographs and conclusions

Indices of disagreement on several other questions are listed in Table 18. The questions were selected with a view to using them for a classification of radiographic findings. They include questions with the lowest and highest levels of disagreement.

Figure 4
Examples of curves of disagreement in radiographic and sputum-smear examinations

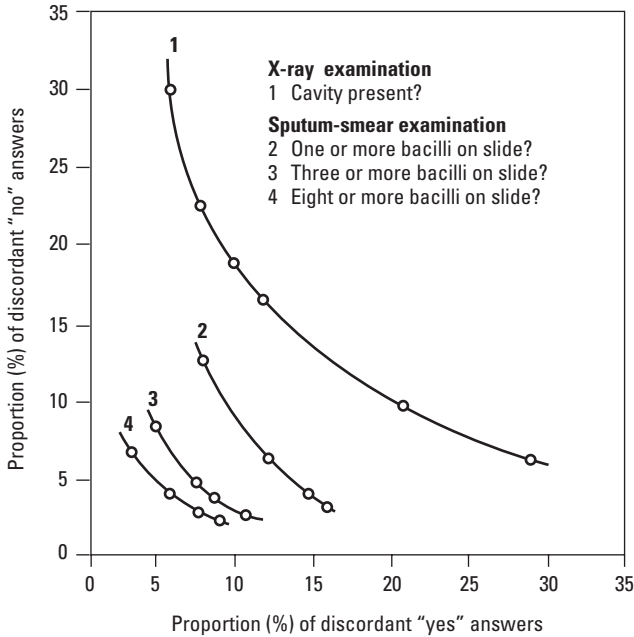


Table 18
IUAT international study on radiographic classification: indices of disagreement on various questions^a

Question	Index of disagreement
Abnormality in lymph nodes?	60
Abnormality in lung, probably tuberculous?	45
Calcification in lung?	42
Non-calcified abnormality, probably tuberculous?	37
Is the film abnormal?	34
Need for medical action?	31
Cavity present?	28

^a Source: reference 14.

The level of disagreement on the question about the presence of any pulmonary abnormality was quite unexpected, as was the poor agreement on the question about calcification. The discrepancies regarding abnormalities of the lymph nodes, including calcifications (one of the most frequently described radiographic findings), were particularly striking. The highest level of agreement – or rather the least disagreement – was seen in response to the question about cavities. This information has to be seen in the context of medical action. Thus 5% of patients with smear-positive tuberculosis were reported as having a normal radiograph, 17% as having some (probably non-tuberculous) abnormality, and 24% as not requiring clinical action for a tuberculous lesion. If treatment had been restricted to patients in whom 50% or more of the readers judged cavitation to be present, only one-third of those with positive sputum smears would have received treatment. On the other hand, among those who were regarded by 50% or more readers as probably tuberculous and in need of treatment, about four or five times as many bacteriologically negative persons as sputum-positive patients would have received treatment (a disproportion similar to that frequently observed in clinics where diagnosis is made merely on radiographic grounds) (16).

Co-morbidity with HIV infection and tuberculosis further diminishes the reliability of chest radiography for the diagnosis of pulmonary tuberculosis. As noted above, extensive inter- and intra-observer variation in chest film interpretation was documented among highly trained and experienced radiologists and chest physicians in the decades before HIV-associated tuberculosis. Since the emergence of HIV/AIDS, clinical studies have consistently documented the atypical radiographic pattern seen in patients with both pulmonary tuberculosis and HIV. Hilar or mediastinal adenopathy, middle or lower lung field infiltrates, and absence of pulmonary infiltrates and cavities are common in such patients (17), as are normal and minimally abnormal chest radiographs (18).

In Malawi, where HIV infection is present in up to 75% of patients with tuberculosis, two hypothetical diagnostic strategies were compared in 402 adults seeking care for symptoms of pulmonary tuberculosis (19). In one strategy, the first diagnostic step would have been chest radiograph, followed by sputum smear examination in patients whose chest films were consistent with tuberculosis. Of 172 patients whose chest films were read as not consistent with tuberculosis, 13 (8%) had AFB smear-positive tuberculosis and 53 (31%) either AFB smear- or culture-positive disease. All these cases would have been missed with this screening strategy. Conversely, of 230 patients with chest films consistent with tuberculosis, who would have been treated for “smear-negative” tuberculosis, 27% had smear- and culture-negative sputum.

In the second strategy, the patients would have first been evaluated with sputum smears, followed by chest radiograph in those who had negative sputum smears. Of 291 patients with negative sputum smears, 159 (55%) had chest radiographs not consistent with tuberculosis and would not have been diagnosed, although 40 (25%) were actually culture-positive. This strategy would therefore have resulted in fewer patients, and none of those with smear-positive tuberculosis would have been missed. However,

chest radiograph as the diagnostic second step for patients with negative smears was not sensitive. Conversely, of the 132 patients with negative smears who had chest radiographs consistent with tuberculosis and who would have been treated with this strategy, 47% had smear- and culture-negative sputum tests.

In summary, the experience of many decades of detailed data collection and analysis indicate that chest radiography for diagnosis or follow-up of pulmonary tuberculosis cases, with or without HIV co-infection, is unreliable (19, 20).

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13. What are the relative merits of chest radiography and sputum examination (smear microscopy and culture) in case detection among new outpatients with prolonged chest symptoms?¹

A. Harries²

This question was one of several investigated in a comprehensive socio-epidemiological study of 2229 randomly selected new outpatients, undertaken by the National Tuberculosis Institute, Bangalore, India (1, 2). The outpatients, who presented with chest symptoms (cough for 2 weeks or more, chest pain and fever for 4 weeks or more, or haemoptysis), were examined radiographically and bacteriologically. A sputum specimen was collected on the spot from each patient and examined by direct smear microscopy and culture. Smear examination was carried out with the Ziehl–Neelsen method while the patient waited. Culture was performed on two slopes of Löwenstein–Jensen medium. All positive cultures were then tested *in vitro* for identification of the organism and drug susceptibility. Experienced technicians at the research laboratories of the National Tuberculosis Institute did the bacteriological work.

Table 19 shows that 227 of the 2229 patients were classified by radiography as tuberculous (and thus in need of treatment), but that 81 of these were not confirmed as tuberculous by bacteriological examination. Among the remaining 2002 patients classified as normal or as having a disease other than tuberculosis, there were 31 in whom tubercle bacilli were found by sputum culture and/or smear microscopy.

Because sputum culture is regarded as the most reliable diagnostic method, a correlation was made between the results of radiography and culture. The data given in Table 20 are identical to those in Table 19, except that the two groups of radiographically normal and non-tuberculous persons were pooled. Taking the results of culture as the criterion of correct diagnosis, 20 (12%) of 162 culture-positive patients would have been missed because they had been misclassified by radiography as normal or non-tuberculous. On the other hand, among the 227 patients classified radiographically as tuberculous, 85 (37%) were not confirmed as such by culture.

The results of direct smear microscopy and culture were also correlated. As Table 21 shows, 32 (20%) of the 162 culture-positive patients would have been missed by direct smear microscopy of a single spot specimen, while 15 (10%) of the 145 smear-positive patients proved to be negative by culture.

¹ Based on the chapter by K Toman in the previous edition.

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Table 19

Results of radiographic examination compared with those of sputum smear microscopy (S) and sputum culture (C) in outpatients with clinical signs suggestive of tuberculosis^a

Classification by radiography	No. of patients	Result of sputum examination			
		S+ C+	S- C+	S+ C-	S- C-
Tuberculosis	227	122	20	4	81
Other abnormal shadows (non-tuberculous)	304	8	4	1	291
Normal	1698	—	8	10	1680
Total	2229	130	32	15	2052

^a Source: reference 2.

Table 20

Correlation of the yield of radiographic examination and sputum culture in patients with clinical signs suggestive of tuberculosis

Radiography	Culture		
	Positive	Negative	Total
Positive	142	85	227
Negative	20	1982	2002
Total	162	2067	2229

Taking the results of sputum culture as the criterion for correct diagnosis, the findings of the study may be summarized as follows: among 162 tuberculosis patients in whom the diagnosis was verified by culture, 32 (20%) would have been missed by smear microscopy and 20 (12%) by radiography.

Among 145 patients positive by smear, 130 (90%) were confirmed by culture. The remainder (10%) gave an apparently false-positive result, because of either a reading error or the presence of artefacts, or because the bacilli seen under the microscope had lost their ability to grow on culture. There are many possible reasons for the latter occurrence. In patients under treatment, the bacilli may have been killed or seriously harmed by effective treatment. In untreated patients, the capacity of tubercle bacilli to grow on cultures may have been impaired, e.g. by exposure of the specimen to heat

Table 21

Correlation of the yields of two cultures and of direct smear microscopy of a single spot specimen in patients with clinical signs suggestive of tuberculosis

Smear	Culture		
	Positive	Negative	Total
Positive	130	15	145
Negative	32	2052	2084
Total	162	2067	2229

or sunlight, long storage, excessive decontamination procedures, or an overheated centrifuge or incubator. However, patients whose first spot specimen is indisputably positive by smear microscopy but negative by culture have a fairly high chance of being positive by both examinations in subsequent specimens (see “What is the additional yield from repeated sputum examinations by smear microscopy and culture?”, page 46). Therefore, if persons with signs and symptoms suggestive of tuberculosis are treated on the basis of properly performed and clearly positive smear microscopy, not confirmed by culture, there is little likelihood of serious over-treatment.

On the other hand, of the 227 patients classified by radiography as “tuberculous and in need of treatment”, a sizeable proportion (37%) did not have tuberculosis confirmed by culture. The patients in this study had been examined because they had symptoms. This proportion is likely to be much higher in populations examined by indiscriminate mass radiography, i.e. irrespective of the presence of symptoms. Several studies have indeed shown that persons with chest radiographic shadows of unknown origin, who have no history of previous tuberculosis and in whom tubercle bacilli cannot be demonstrated by smear microscopy and/or culture, particularly when repeated, are in fact rarely true cases of tuberculosis. Follow-up studies (3–6) have demonstrated that, although there is a greater likelihood of such persons becoming culture-positive than those with a normal radiograph, only a small proportion (0.4–4.8%) actually did so within the first year of observation. The risk declined in subsequent years.

Treating persons with chest film shadows of unknown origin as a matter of routine would therefore be to treat many, or even most, of them unnecessarily or wrongly.

In areas with a high prevalence of HIV, it is also inappropriate to screen persons suspected of having tuberculosis with chest radiography. In a study of 402 suspected tuberculosis cases in Malawi (7), 230 patients had chest films thought to be typical of tuberculosis. Sputum smears were positive in 43% of these patients and sputum cultures were positive in 71%. On the other hand, in 172 patients with a normal chest

film or with an abnormal chest film not typical of tuberculosis, 13 (8%) were sputum smear-positive and 49 (28%) were culture-positive. Screening by chest radiography, followed by sputum smears in those with chest films typical of tuberculosis, was more costly and less sensitive than screening by sputum smears and performing chest radiography only in those with negative results on sputum smear examination.

Some technical and operational aspects of chest radiography, sputum culture and smear microscopy

Screening by radiography alone, even in areas of sub-Saharan Africa with high HIV prevalence, will result in a high rate of over-diagnosis. Moreover, the operational shortcomings of radiography in developing countries are considerable. X-ray units are expensive and their operation requires specially trained technicians. There are frequent prolonged interruptions due to the breakdown of equipment, lack of spare parts and repair facilities, scarcity of films, or unreliability of the electricity supply. Another operational disadvantage is that the results of radiographic examination are commonly available only after 2–3 days, and sometimes later. A sizeable proportion of patients do not return to the centre for their results, and efforts to retrieve them are often unsuccessful.

As a diagnostic method, sputum culture is known to be more sensitive than smear microscopy. It differentiates tubercle bacilli from other microorganisms and therefore provides definite identification of the bacilli. The technical superiority of culture over smear microscopy is largely due to quantitative factors. Whereas the amount of sputum on a smear is 0.01 ml (see “How many bacilli are present in a sputum specimen found positive by smear microscopy?”, page 11), the size of an inoculum for culture is usually 0.1 ml, i.e. about 10 times as much. Moreover, usually only about 1% of the smear (100 oil-immersion fields) is examined by bright-field microscopy, whereas in the culture test-tube the whole yield of colonies may be seen practically at a glance. Although a large proportion of organisms are destroyed by decontamination procedures, the quantitative differences are still so large that the probability of finding bacilli by culture is higher than by direct smear microscopy. This is an obvious advantage in cases where a specimen contains only small amounts of acid-fast bacilli (see “How reliable is smear microscopy?”, page 14).

Unfortunately, culture has a number of disadvantages, mainly of an operational nature. The method requires specially trained and skilled personnel, of whom there is a shortage in most developing countries. There is also a need for special facilities and equipment, a permanent supply of water and electricity, and reliable thermo-regulation of the hot room. In hot and humid climates particularly, air-conditioning facilities and special air filters are needed to prevent airborne contamination of cultures. Properly ventilated inoculation cabinets and other safety measures must be provided. For all these reasons, culture methods are practicable in only a few laboratories in developing countries.

One of the greatest shortcomings of sputum culture is the long interval before results become available: 4–6 weeks or more with solid media. In developing coun-

tries, this delay leads to many patients being “lost”. They may never return to the health centre and often cannot be traced. Thus the benefits of culture are often outweighed by the losses occasioned by the long wait for results, and the higher sensitivity is largely counterbalanced by operational disadvantages of culture.

Direct smear examination certainly has a number of technical shortcomings, but its operational advantages are obvious. It is relatively easy to perform, much less expensive than radiography or culture, and does not require highly specialized personnel. The fact that the diagnosis of tuberculosis in persons discharging large amounts of bacilli may be established and treatment started on the same day is without doubt the greatest operational advantage of smear microscopy. It reduces to a minimum “losses” of patients due to long waiting periods, and it is also the only diagnostic method practicable almost everywhere. For these reasons, case detection and diagnosis in high-prevalence countries will have to rely on this method for some time to come.

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14. How does pulmonary tuberculosis develop and how can it be detected at an early stage?

*K. Toman*¹

On clinical and epidemiological grounds, emphasis has rightly always been laid on early diagnosis of tuberculosis. However, this has led to a certain confusion. Early or incipient tuberculosis has frequently been considered to be synonymous with minimal tuberculous disease. Likewise, advanced tuberculosis has usually been regarded as synonymous with old or chronic tuberculosis. Yet the terms “early”, “incipient”, “chronic”, and “old” are strictly and exclusively concerned with time, while “minimal”, “moderate”, and “advanced” indicate merely the extent of the disease, i.e. the volume of lung tissue involved. Terms such as “early” and “minimal” have by no means a constant relationship, nor are they necessarily linked. In fact, a lesion of a few months’ duration may be minimal or far advanced, and the extent of a lesion gives surprisingly little indication of its duration.

The age of a fresh lesion can be estimated only when radiographic evidence of a previously normal lung is available. A few longitudinal radiographic surveys demonstrate the history of early tuberculosis.

In one such survey in a population of about 100 000 in Kolín, Czechoslovakia,² persons aged 14 years and older were screened repeatedly.¹ The study (1, 2) lasted 12 years, during which each member of the eligible population was X-rayed five times, at 2- or 3-year intervals. Between radiographic rounds, the search for cases was continued by the local health services, which patients attended because of symptoms or for regular check-up. Each film was carefully filed and could therefore always be compared with the most recent film. Thus, it was possible to determine the period within which a new lesion had developed. The films were read independently by two readers and an umpire reader (Table 22).

In 165 persons in whom tuberculosis had been newly detected and whose previous radiographs had been normal, the time between the last normal chest radiograph and the first abnormal one was measured. Patients were grouped into three categories: those who were sputum smear-positive as well as culture-positive, those who were

¹ Deceased.

² Country name valid at the time of the study cited.

CASE DETECTION

Table 22
Interval between last normal chest radiograph and diagnosis of pulmonary tuberculosis^a

Interval (months)	Number of smear+, culture+	Cumulative %	No. of smear-, culture+	Cumulative %	Number diagnosed at autopsy
≤12	8	16	14	14	6
<24	18	52	39	53	7
<36	16	84	31	86	1
≥37	8	100	14	100	3
Total	50		98		17

^a Source: references 1, 2.

smear-negative but culture-positive, and those in whom the diagnosis had first been made at autopsy and verified by bacteriological examination (1, 2).

Table 22 shows that, within 12 months, 28 bacteriologically positive cases had developed. Surprisingly, a significant proportion of these already had advanced tuberculosis with positive sputum by direct smear microscopy. Even more striking was the finding that six cases had developed so fast that they were found only at autopsy – less than 12 months after the last normal chest radiograph. (In some of these cases, tuberculosis had been identified by the pathologist as the leading cause of death.) During the second part of the Kolín study (1965–1972), 10 previously unknown cases of new tuberculosis were first diagnosed at autopsy – some of them shortly after a normal radiograph. These 10 cases constituted one-quarter of all deaths from tuberculosis during that period – and that in spite of systematic and intensive screening of the entire population. The actual number might well have been even higher, because only one-quarter of all persons who died in the study area (mainly those who died in hospital) were subjected to necropsy (2).

The data presented in Table 22 might perhaps be biased, since the interval between radiographic rounds was never less than 2 years and was usually 3 years. In this regard, very instructive data are available from Japan, where mass radiography of the population has been carried out at yearly intervals (3). Similar data are also available from an epidemiological study (4) in Niigata Prefecture, Japan (population 2 350 000).

As discussed elsewhere (see “What is the role of case detection by periodic mass radiographic examination in tuberculosis control?”, page 72), more than half of the cases positive by direct smear microscopy had developed tuberculosis within 12 months of the last normal radiograph. Since only one-fifth of the new cases had been detected by mass radiographic surveys, the majority (72%) were found by the health services, where patients attended mainly because of symptoms. The same applied to new patients who were positive by culture only (4).

The conclusions to be drawn from the findings of the above-mentioned studies are the following:

- A large proportion of new cases, starting in a normal lung, developed within months.
- Even cases already so advanced that they were discharging large numbers of bacilli demonstrable by microscopy, and were probably cavitory, developed rapidly.
- A case of advanced smear-positive tuberculosis, when seen for the first time, is not necessarily old or chronic: it may well be as recent as a case with minimal lesions that is positive by culture only.
- Both types of disease – advanced, smear-positive tuberculosis and minimal, only culture-positive tuberculosis – developed within the same time. Thus it is likely the smear-positive cases developed so fast that they did not pass through a perceptible minimal phase. Rapid development of disease should not be confused with so-called “galloping consumption”. This form of fulminant tuberculosis, running an exceptionally rapid course, has been described in the older literature and observed occasionally in individuals who are under extraordinary stress and physical vulnerable.

Every experienced clinician knows that, after a few days of sometimes vague or uncharacteristic complaints (and an initially normal chest radiograph), tuberculous pleuritis with an effusion of as much as 1 litre can appear suddenly; or that sometimes an extensive pneumonic lesion may develop in an initially normal lung within a few days and cavitation may take place within a week (5).

In a study in the USA of 1000 patients with early tuberculosis (6, 7), the authors concluded that:

- Sudden symptomatic onset of pulmonary tuberculosis is not less frequent than insidious onset.
- The extent of the lesion does not bear a direct relation to the duration of the disease (of all patients reaching an advanced stage of the disease, the majority do so within the first 6 months).
- Cavitation is not a late occurrence: its frequency is nearly the same at all temporal stages of the disease.

Another investigation determined the intervals at which persons at special risk (e.g. contacts) should be re-examined to discover all cases at the earliest possible stage (5). Such persons were usually examined at yearly intervals; however, one series conducted examinations every 6 months and another did so at shorter intervals. In none of these series were all cases diagnosed with minimal lesions. Even with examinations at 4-monthly intervals, 21% of patients were found to be in the moderately advanced stage and a smaller proportion even at the far-advanced stage of the disease. The conclusion was that a 6-monthly interval between examinations is too long to prevent the occurrence of advanced, severe cases.

However, even in the most affluent countries, screening the adult population by indiscriminate radiography at intervals shorter than 12 months has not been found practicable. Moreover, as data from Japan have shown, even if more frequent examination were feasible, a large proportion of cases of bacteriologically verified disease would be found at an advanced, smear-positive stage. It is obvious that mass radiography will fail to detect the majority of cases soon after the onset of disease.

Incipient tuberculosis and symptoms

It is sometimes asserted that screening of a population by mass radiography is essential because about half of all new patients have no symptoms (2). The literature on this issue is extensive. It is well known that the taking of case histories is arbitrary and unreliable, and the fact that many new patients are found by mass screening of apparently healthy individuals does not necessarily mean that these people have no symptoms. Likewise, when a new patient is asked about the presence of symptoms and gives a negative answer, this should not be taken as objective evidence of the absence of symptoms.

Few of the prospective studies reported so far have been designed in such a way as to eliminate bias, at least to a large extent. Some were designed by sociologists and carried out by specially trained personnel using a standardized interviewing technique according to a protocol. In one series of studies, persons were examined and interviewed in parallel without knowing the results of their examinations (8–13). In socio-epidemiological studies of a poor rural population in India in the early 1960s, it was found that 95% of patients who were positive by direct smear microscopy were aware of one or more symptoms suggesting tuberculosis. About 70% complained of cough as the leading symptom, while the rest gave greater importance to other complaints (8, 14). About two-thirds had symptoms of only 1–3 months' duration (9, 10). This was surprising in a population believed to be unaware of symptoms.

In another prospective study on case detection in a population of about 6 million, some 1600 smear-positive patients were interviewed about symptoms (11). The study was carried out in parallel to the Kolín study (see Table 22) as one of the projects of the Tuberculosis Surveillance Research Unit, and the two study populations were of similar composition. The results were strikingly similar to those of the above-mentioned studies: 73% of patients complained of cough, ranking it first or second in importance as a symptom. The remaining 20% complained of fever or an influenza-like illness, and only 7% denied having any subjective symptoms. The duration of symptoms was also similar, with 62% having had symptoms for less than 3 months and 83% for up to 6 months.

It thus seems that symptoms are present in more than 90% of patients with sputum positive by direct smear microscopy, and that these symptoms are apparent in the early phase of the disease.

The question may arise as to whether the development of less serious disease – with sputum negative by smear and positive by culture only – is asymptomatic. In the

socio-epidemiological study carried out in India (8), 54% of the smear-negative, culture-positive patients had one or more symptoms suggesting tuberculosis. In the longitudinal survey carried out in Kolín during the period 1961–1964 (1), 91 (51%) of 180 new patients positive only by culture had symptoms. In the Niigata study (4), 63 (57%) of 109 persons positive only by culture had symptoms that, in four-fifths of cases, had lasted less than 3 months.

Since more than 90% of infectious patients develop perceptible symptoms within a few weeks of the onset of tuberculosis, early detection is possible – not by traditional mass radiography, but also by sputum examination of symptomatic persons. Mass radiography would detect most of these cases only 1–3 years after the onset of the disease (14), by which time most of the harm had already been done to the community (see “What is the role of case detection by periodic mass radiographic examination in tuberculosis control?”, page 72).

The WHO Expert Committee on Tuberculosis emphasized the importance of case detection among patients with symptoms at its eighth and ninth meetings (13, 15). The Committee also stressed the need to increase the awareness of symptoms suggestive of tuberculosis in the community and among all health workers. Patients with cough of several weeks' duration should have their sputum examined by microscopy as the first priority for case detection. If found to be sputum-positive, these patients are the first priority for treatment.

The search for patients positive only by culture is of secondary epidemiological importance. Patients without symptoms are not an urgent public health concern. Their prognosis is likely to be favourable and their infectiousness, if any, is slight. Indeed, it has been proved that there is practically no transmission of infection if patients do not have cough (16).

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15. What is the role of case detection by periodic mass radiographic examination in tuberculosis control?¹

*H. Rieder*²

Case detection means the early detection of individuals discharging and transmitting tubercle bacilli. But case detection is not an end in itself: it is carried out in order to treat the sources of infection so as to alleviate their suffering and to render them non-infectious.

The capacity of excretors of tubercle bacilli to infect others varies considerably. Bacteriological and epidemiological studies have revealed fundamental differences in the degree of infectiousness of different categories of patients with pulmonary tuberculosis.

A comprehensive study was made of the bacterial content of pulmonary lesions in patients who had never been given treatment (1). The investigators found that the number of tubercle bacilli in the various types of lesions varied substantially. In an encapsulated, solid nodule, 2 cm in diameter, having no communication with the bronchi, the number of bacilli ranged from about one hundred (10^2) to not more than a few thousand (10^4). In contrast, a cavitary lesion of the same extent might contain about 10 million to a billion bacilli (10^7 – 10^9), i.e. 100 000 times as many as in non-cavitary lesions. Such enormous quantities of tubercle bacilli discharged with the sputum can invariably be demonstrated by simple smear microscopy, while the small numbers coming from non-cavitary lesions are often demonstrable only by culture or amplification techniques.

This may explain why patients with non-cavitary tuberculosis, negative sputum smears, and positive sputum cultures have a comparatively favourable clinical prognosis. They are more likely to undergo spontaneous healing than patients with cavitary lesions discharging large numbers of tubercle bacilli demonstrable by direct microscopy. By the same token, patients with different bacteriological sputum status – discharging small or large quantities of tubercle bacilli – do not have the same epidemiological relevance.

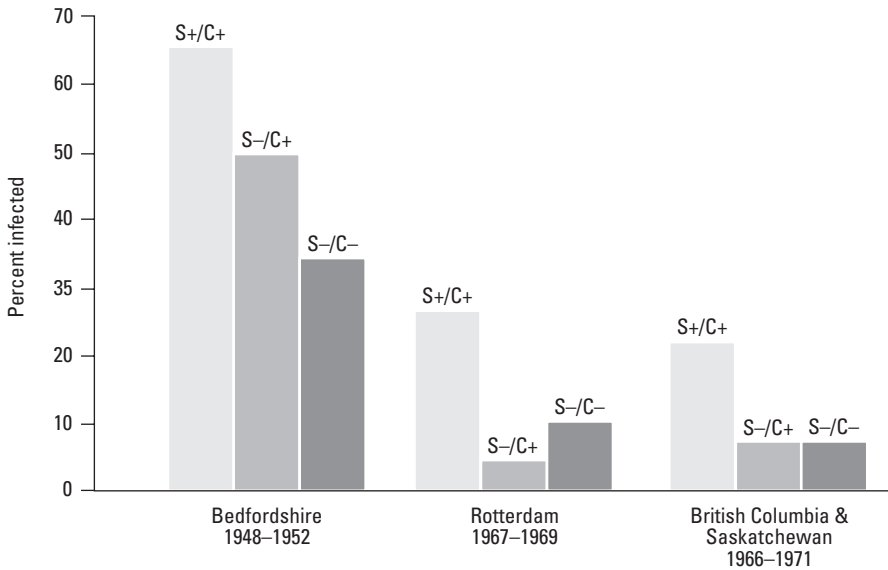
Several experimental (2) and epidemiological (3–5) studies have examined the

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*Figure 5***Risk of infecting contacts according to the bacteriological status of the pulmonary TB source**

(S+/C+ indicates contacts of smear- and culture-positive cases, S-/C+ contacts smear-negative and culture-positive case, and S-/C- contacts of smear and culture-negative source cases.)



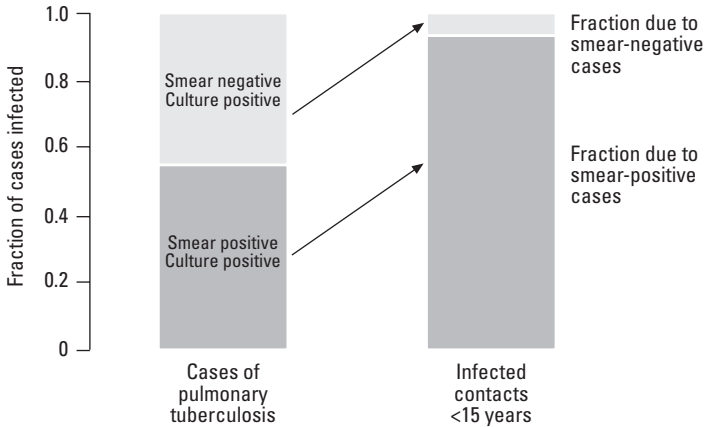
^a Source: references 4-6.

relationship between the frequency of infection and the bacteriological status of the source of infection. Figure 5 summarizes the major findings from three epidemiological studies. In each of the studies, children under the age of 15 years who were contacts of tuberculosis cases were tested with tuberculin and grouped according to the source case to which they had been exposed.

The absolute differences between the studies are not important (they were done at different times in different settings and probably used different definitions of contact). What matters is that all the studies clearly demonstrate that smear-positive cases are the major sources of infection: the highest prevalence of tuberculous infection was consistently found among children who had been exposed to sputum smear-positive tuberculosis cases.

From the study in British Columbia and Saskatchewan (5), the proportion of infections attributable to sputum smear-positive cases could be estimated as shown in Figure 6. In this setting, more than 90% of all infections were attributable to smear-positive source cases. Similarly, a recent study from San Francisco, USA, using

Figure 6
Proportion of transmission attributable to smear-positive and to culture-only-positive pulmonary tuberculosis in British Columbia and Saskatchewan^a



^a Source: reference 6.

molecular epidemiological techniques, showed that more than 80% of all transmission was attributable to smear-positive cases (6).

Once the category of sputum smear-positive patients had been shown to be the most significant epidemiologically, the questions that arose were: “How are infectious patients currently being discovered?” and “What is the contribution of mass radiography to the detection of this high priority group?”

Detection of sputum smear-positive tuberculosis cases: results of mass radiography

Table 23 summarizes the results of WHO-assisted investigations, mainly carried out in cooperation with the Tuberculosis Surveillance Research Unit (7). In all the countries participating in this investigation, mass radiography for tuberculosis case detection had been a routine procedure for about 20 years.

As Table 23 shows, mass radiography made a surprisingly small contribution to the detection of smear-positive cases. The majority of cases were discovered by other means, mostly through people seeking medical help on their own initiative because of symptoms.

Another important observation from the study projects, particularly in the Netherlands and in the Kolín study, carried out in areas with populations of about 100 000, was that the proportion of smear-positive cases among those with newly detected tuberculosis had changed little despite intensive mass radiography carried out at 2–3-year intervals. Thus, in the Netherlands, although the annual incidence rates of tuberculosis were steadily decreasing, the proportion of smear-positive cases remained

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Table 23
Mode of detection of sputum-positive tuberculosis cases^a

Project	Study period	No. of smear-positive cases	Mode of detection		
			Mass radiography (%)	Symptoms (%)	Other (%)
Saskatchewan	1960–1969	265	12	66	22
Ontario	1967–1968	632	15	66	19
Kolín	1965–1972	132	23	54	23
Netherlands	1951–1967	9301	25	39	36

^a Source: references 8, 9.

Table 24
Smear-positive cases detected in Kolín district, Czechoslovakia,^a 1965–1972^b

Method of detection	1965	1966	1967	1968	1969	1970	1971	1972	Total
Symptoms	16	6	7	9	8	7	8	10	71
Mass radiography	–	14	–	–	11	1	–	4	30
Other	6	4	2	5	3	3	4	4	31
Total	22	24	9	14	22	11	12	18	132

^a Country name valid at the time of the study.

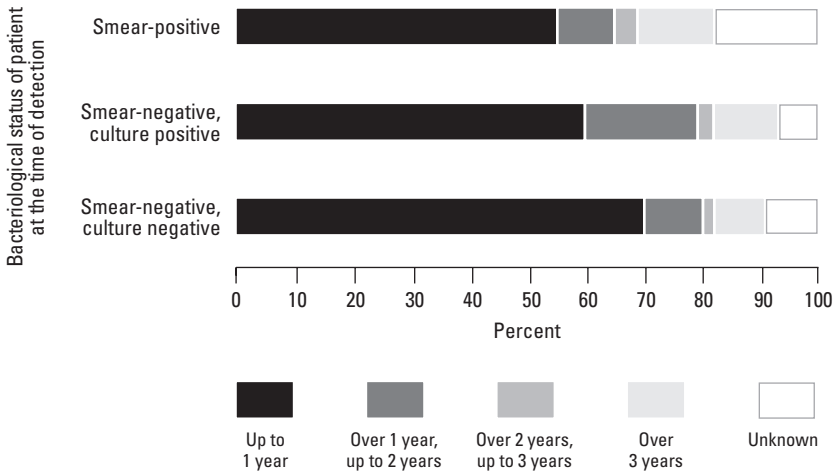
^b Source: reference 9.

unchanged during the observation period (1951–1967). Of every 100 new patients discharging tubercle bacilli, 46 were smear-positive, despite 17 years of mass radiography at 3-year intervals (7).

An equally puzzling observation emerged from the Kolín study (Table 24). The proportion of new smear-positive cases was not substantially influenced by repeated surveys with 95% coverage of the eligible population aged 14 years and above (8). Three-quarters of these new cases developed in persons who had had a normal chest radiograph at the previous survey. The possibility that these advanced cases had developed from pre-existing, but overlooked, lesions could safely be ruled out. Two readers and one referee assessed each radiograph independently, and a WHO expert also examined a random sample of the films. For each new case in which there was an abnormal finding, all the previous films were carefully scrutinized to ascertain whether any radiographic lung shadow had existed before but had been missed or misinterpreted.

Figure 7
Interval between the last normal radiograph and the development of tuberculosis in persons of differing bacteriological status (Niigata, Japan)^a

Each bar represents all cases with the bacteriological status indicated at the time of detection.

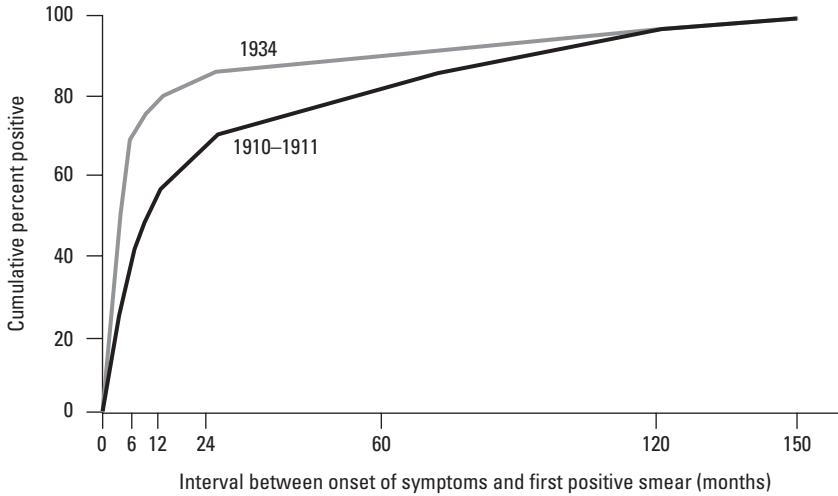


^a Source: references 8–10.

All cases of tuberculosis and all individuals with abnormal chest radiographs discovered in any of the surveys were either treated or closely followed up. It was therefore expected that new cases discovered in subsequent surveys would be mainly in persons with previously normal radiographs. Thus they would be found at a very early stage – at worst when the sputum was positive by culture only. Yet, year after year, a considerable proportion of the newly diagnosed cases already had advanced smear-positive tuberculosis. Since in the majority of cases (about 75%) no detectable lung lesion had existed at the previous examination, the only possible conclusion was that most of the new cases with smear-positive tuberculosis must have developed rapidly. This conclusion was tested and confirmed (8–10); see Figure 7.

Figure 7 shows that more than 50% of the newly discovered, smear-positive cases developed in less than 1 year. The cases positive by culture (usually with minimal lung lesions) developed within the same time. It looks as if new tuberculous lesions develop either slowly or quickly, right from the beginning. For reasons not yet fully known, tubercle bacilli grow very slowly in certain lesions and thus are present only in small numbers, but multiply rapidly in other lesions, reaching enormous counts within a few weeks. It would therefore be wrong to regard all patients with cavitory, smear-positive tuberculosis as old or chronic cases due to delayed diagnosis, because of the patient's or doctor's negligence. As Figure 7 shows, smear-positive tuberculosis of the

Figure 8
Cumulative percentage of sputum smear-positivity after onset of symptoms among patients who were ultimately smear-positive, Sweden 1910–1911 and 1934^a



^a Source: reference 11.

lung can be as old or as recent as a small lung lesion positive only by culture, since at least half of such cases developed in less than 1 year in apparently healthy persons with normal radiographs.

Data from the Kolín study also showed that about four-fifths of new patients who had normal chest radiographs at the previous mass radiography developed the disease within 3 years. Thus it appears that mass radiography, carried out at intervals of 3 years, may to a large extent fail to detect cases sufficiently soon after the onset of disease. Even intervals of 6 months may be too long, as a Swedish study has shown (11). Progress from onset of symptoms to sputum smear-positive tuberculosis was very rapid in a large proportion of patients (Figure 8).

Conclusions

It has been proved that the early detection of all cases with smear-positive pulmonary tuberculosis – the most dangerous sources of infection – by means of periodic mass radiography is impractical, even when such radiography is repeated at short intervals (12). The great majority of sputum smear-positive cases develop in a shorter time than the shortest practical interval between two mass radiography survey rounds. Moreover, 90% of patients with rapidly progressive pulmonary tuberculosis have objective symptoms, such as cough, fever, loss of weight, sputum, and haemoptysis (13). These symptoms develop rather soon after the onset of the disease, prompting the patient

to seek medical advice. Most smear-positive tuberculosis cases are therefore not detected in periodic case-detection campaigns but rather by regular health services that patients can consult whenever they feel ill.

For these reasons, mass radiography is not a recommended case-detection method. Additional obstacles to the effective operation of periodic mass radiography are the lack of good roads, the high breakdown rate of vehicles and X-ray machinery, and the high cost and scarcity of spare parts and repair facilities.

In its ninth report (14), the WHO Expert Committee on Tuberculosis noted that "mass miniature radiography is a very expensive screening procedure for tuberculosis, even when the prevalence is high. Other disadvantages of mass radiography are as follows:

1. It contributes only a small proportion of the total number of cases found.
2. It has no significant effect on the occurrence of subsequent smear-positive cases, as they usually develop so rapidly that they arise between the rounds of mass radiography examinations (thus it follows that case-detection and treatment facilities should be constantly available for an indefinite period to come).
3. It requires the services of highly qualified technicians and medical staff who could be better used in other health service activities.
4. The apparatus or the vehicles used to transport it are often out of service because of mechanical breakdown for months on end, especially where spare parts are in short supply.

The Committee concluded that the policy of indiscriminate tuberculosis case-finding by mobile mass radiography should now be abandoned."

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16. How does the diagnosis of tuberculosis in persons infected with HIV differ from diagnosis in persons not infected with HIV?

A. Harries¹

Pulmonary tuberculosis is the most common manifestation of tuberculosis in adults infected with HIV. Tuberculosis occurs at various stages of HIV infection, with the clinical pattern correlating with the patient's immune status. In the early stages of HIV infection, when immunity is only partially compromised, the features are more typical of tuberculosis, commonly with upper lobe cavitation, and the disease resembles that seen in the pre-HIV era. As immune deficiency advances, HIV-infected patients present with atypical pulmonary disease resembling primary tuberculosis or extra-pulmonary and disseminated disease, commonly with hilar adenopathy and lower lobe infection (1).

Diagnosis of pulmonary tuberculosis

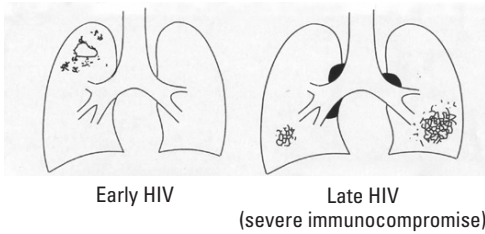
Clinical features in pulmonary tuberculosis are generally similar in HIV-infected and HIV-negative patients. However, cough is reported less frequently by HIV-infected patients, probably because there is less cavitation, inflammation, and endo-bronchial irritation as a result of a reduction in cell-mediated immunity (2). Similarly, haemoptysis, which results from caseous necrosis of the bronchial arteries, is less common in HIV-infected patients.

Tuberculin skin tests have limited value for individual adult diagnosis, although they are useful for measuring the prevalence of tuberculous infection in a community. In the presence of active tuberculosis, the tuberculin skin test may be negative. For example, one study of HIV-positive pulmonary tuberculosis patients in Zaire found cutaneous anergy in 8% of those with a CD4-lymphocyte count >500/μl and 54% in those with a CD4-lymphocyte count <200/μl (3).

Sputum smear microscopy remains the cornerstone of tuberculosis diagnosis, even in areas of high HIV prevalence. Systematic studies in sub-Saharan Africa have shown that most HIV-infected pulmonary tuberculosis patients are sputum smear-positive, although the proportion of patients with smear-negative, suspected pulmonary tuberculosis is greater in HIV-infected than in HIV-negative tuberculosis patients (1, 4).

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Figure 9
Radiographic findings in tuberculosis patients with HIV infection



HIV-infected, smear-positive patients also tend to excrete significantly fewer organisms in sputum than HIV-negative patients, which can lead to acid-fast bacilli being missed if insufficient high-power fields are examined by microscopy.

Chest radiography is needed for persons who are suspected of having tuberculosis, are sputum smear-negative, and do not respond to a course of broad-spectrum antibiotics. Bronchitis and pneumonia with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and other common pathogens are frequent in HIV-infected persons. No radiographic pattern is diagnostic of tuberculosis, although the classical hallmarks of the disease are cavitation, apical distribution, pulmonary fibrosis, shrinkage, and calcification. HIV-infected patients with relatively well-preserved immune function will often show these typical features; as immunosuppression worsens, however, chest radiographs more often show atypical features such as pulmonary infiltrates affecting the lower lobes and intrathoracic lymphadenopathy. Sometimes the chest radiograph is normal (1, 4): in one study in the United States of America, 21% of smear- and/or culture-positive tuberculosis patients with a CD4-lymphocyte count <200/ μ l had normal chest radiographs (5).

Diseases other than tuberculosis can cause both the classical and atypical chest radiographic features. If sputum smears are negative, other conditions have to be considered in the differential diagnosis.

Important HIV-related pulmonary diseases that may be confused with pulmonary tuberculosis include bacterial pneumonia, *Pneumocystis carinii* pneumonia, Kaposi sarcoma, fungal infections, and nocardiosis.

Extrapulmonary tuberculosis

The main manifestations of extrapulmonary tuberculosis in HIV-infected patients are lymphadenopathy, pleural effusion, pericardial effusion, and miliary tuberculosis (1, 4). The definitive diagnosis of extrapulmonary tuberculosis is often difficult because of the scarcity of diagnostic facilities: in the United Republic of Tanzania, only 18% of patients with extrapulmonary tuberculosis had laboratory confirmation of the diagnosis (6).

Presentation of extrapulmonary tuberculosis in HIV-infected patients is generally no different from that in HIV-negative patients. However, HIV-related tuberculosis lymphadenopathy can occasionally be acute and resemble an acute pyogenic bacterial infection. Diagnosis can be made using simple techniques such as needle aspiration, inspection of lymph nodes biopsies for macroscopic caseation, and examination of direct smears from the cut surface. In tuberculous meningitis, the cerebrospinal fluid may be completely normal in HIV-infected patients. Disseminated tuberculosis may be extremely difficult to diagnose. In Côte d'Ivoire, for example, the condition was found in 44% of patients with HIV wasting syndrome who came to autopsy; the diagnosis had not been made ante mortem (7). Bacteraemia with *Mycobacterium tuberculosis* may not be uncommon, and may be accompanied by cough and abnormal chest radiographs in less than half of cases (8–10). Pericardial tuberculosis is not rare and may be diagnosed presumptively from the characteristic balloon-shaped appearance of the cardiac shadow on chest radiography.

Diagnosis in childhood tuberculosis

As in adults, pulmonary tuberculosis is the most common manifestation of tuberculosis in HIV-positive children. The diagnosis of pulmonary tuberculosis in children under 4 years old has always been difficult, and HIV infection further compounds this diagnostic challenge. There is a high incidence of cutaneous anergy in HIV-positive children with tuberculosis, and most cases are diagnosed according to nonspecific clinical and radiographic criteria. Because it is often difficult to distinguish HIV-related pulmonary disease from pulmonary tuberculosis, childhood pulmonary tuberculosis is probably over-diagnosed in many areas.

Implications of diagnostic difficulties

The advent of HIV has made the diagnosis of tuberculosis more difficult, and false diagnoses of tuberculosis probably occur frequently among patients affected by other HIV-related illnesses. Although little has been done to solve or even delineate this important problem, these false-positive diagnoses generally account for only a small proportion of all forms of tuberculosis notified, and thus do not negate the huge increases observed in tuberculosis notifications in HIV-endemic areas. Sputum smear remains the cornerstone of diagnosis, identifying infectious patients so that transmission can be stopped.

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17. What is the role of tuberculin skin testing in the diagnosis of tuberculosis?

*D. Menzies*¹

The tuberculin skin test seems attractive because it involves low technology, is inexpensive, and is relatively easy to administer and read. However, its interpretation remains a subject of controversy and misunderstanding, some of which arises from widely discrepant findings reported in different studies. This wide variation in results does not reflect variation in sensitivity or precision of the test. Rather, the differences reflect considerable variation in the prevalence of true-positive and true-negative as well as in the occurrence of false-positive and false-negative results in different populations.

For the diagnosis of tuberculosis infection, the tuberculin test is practically the only tool currently available. Its usefulness depends on the clinical situation and population, as well as on the availability of resources to manage tuberculin reactors. The tuberculin test is useful for identifying individuals at high risk of disease, such as those with HIV infection or close contacts of infectious tuberculosis patients, who would benefit from treatment of latent tuberculosis infection (see “What is the role of treatment of latent tuberculosis infection in a tuberculosis control programme?”, page 220).

However, the test is not useful for the diagnosis of tuberculosis disease, and will often be misleading because of false-negative and false-positive results. At the time of diagnosis, the tuberculin test is falsely negative in 10–47% of patients with active disease (1–4). The likelihood of false-negative reactions increases with the extent of disease and the age of the individual. Interestingly, this tuberculin anergy appears to be temporary; more than 95% of patients tested after 1 month or more of treatment will have a positive test result (5). In the presence of co-infection with HIV, a far higher proportion of patients with active disease will have a false-negative test result. This is related to the degree of immunosuppression: a false-negative tuberculin test will be seen in 30% of patients with a CD4 T-lymphocyte cell count of $>500/\mu\text{l}$, compared with close to 100% of patients with a CD4 T-lymphocyte cell count of $<200/\mu\text{l}$ (6, 7).

Tuberculin testing is a nonspecific measure of prior mycobacterial sensitization. It may be positive in individuals who have had prior BCG vaccination – although, if

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BCG is given in infancy, a reaction of more than 10 mm is rare after the age of 5 years (8). In persons vaccinated at an older age, such as in primary school, 15–25% will remain positive for as long as 20–25 years (8–10). A false-positive test also commonly results from cross-reacting sensitivity to non-tuberculous mycobacterial antigens (11), which are very common in tropical and subtropical climates (5, 12, 13).

The most important limitation of the tuberculin test for diagnosis of disease is its inability to distinguish latent, or dormant, infection from infection associated with active disease. If a test is used to investigate patients with respiratory symptoms, a positive result only marginally increases the probability that the patient has tuberculosis. This is because, even among patients with a positive test resulting from true tuberculosis infection, the vast majority will not have active tuberculosis disease at the time of assessment.

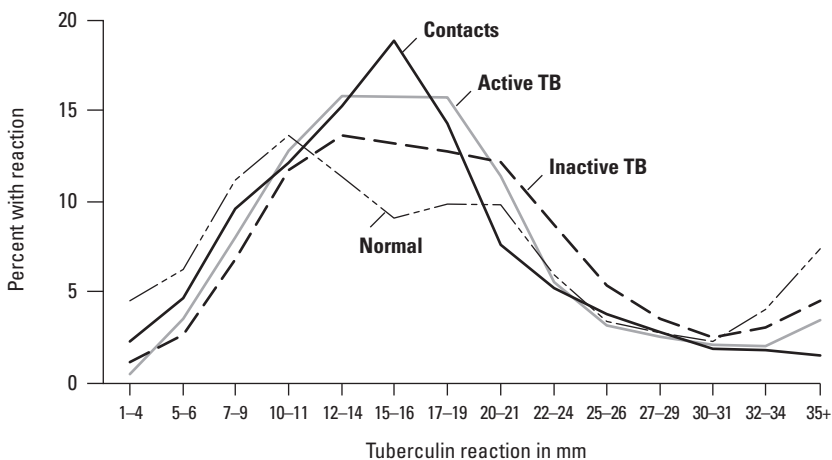
In screening situations, the tuberculin test is even less useful for detecting disease. In situations of high prevalence of tuberculosis, such as in developing countries, a negative test may be misleading.

Does the size of reaction help in distinguishing infection from disease?

Large tuberculin reactions are always more impressive to patients and health care providers alike. It is a common misconception that larger tuberculin reactions are more likely to indicate active disease. As Figure 10 shows, this is not the case. It is true

Figure 10
Pattern of tuberculin reactions in patients with active and inactive tuberculosis, in contacts of active cases, and in normal individuals^a

Curves smoothed by moving three-point average. Tuberculin reactions of 0 mm not shown.



^a Adapted from reference 1.

that the likelihood of disease will vary depending on whether the tuberculin test is more or less than 5 mm. However, beyond 5 mm, the size of the reaction does not distinguish in any way between those with active tuberculosis disease, inactive tuberculosis (abnormal chest radiograph), recent infection (close contacts), or remote infection. Therefore, beyond a certain threshold, size does not matter in the interpretation of a tuberculin reaction (1).

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18. What is the current and potential role of diagnostic tests other than sputum microscopy and culture?

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The sputum smear examination for acid-fast bacilli (AFB) is a low-cost, highly specific means of identifying the infectious sources of spread of tuberculosis. However, it has limitations in that it is relatively labour-intensive. If there is a lack of training, motivation, time, or supervision of laboratory technicians, performance of the AFB smear under programme conditions may be far below its potential. In Africa, where tuberculosis caseloads in some countries have increased 2–4-fold, as many as one in four patients diagnosed as having smear-negative tuberculosis actually have positive smears (1). Furthermore, the AFB smear requires two visits to the health facility by the patient, and will not, even when optimally performed, identify patients with small numbers of bacilli in their sputum that are positive by culture only (see “What is the role of mycobacterial culture in diagnosis and case definition?”, page 35). Although these patients contribute only a small proportion of transmission and of mortality due to tuberculosis, their diagnosis and management may absorb a significant proportion of the effort of the clinical and public health staff responsible for tuberculosis control. A rapid, low-cost test that is simpler to perform than the AFB smears and/or that can identify smear-negative and extrapulmonary tuberculosis is therefore desirable. Chemical tests used to suggest a diagnosis of extrapulmonary tuberculosis (e.g. adenosine deaminase, tuberculo-stearic acid) are not considered here.

Immunological tests

The tuberculin skin test

The tuberculin skin test has been in clinical use for more than 90 years (see “What is the role of tuberculin skin testing in the diagnosis of tuberculosis?”, page 84). However, it does not distinguish between tuberculosis infection and disease, and some patients with tuberculosis disease initially have negative tuberculin tests. The test therefore has little role in the diagnosis of tuberculosis disease in adults.

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Serology

The term “serology” refers here to the measurement of humoral (antibody – most commonly IgG) response to *Mycobacterium tuberculosis* through blood tests. Robert Koch introduced the first serological test in 1898, yet after more than a century of development, no currently available serological test offers adequate sensitivity and specificity. This is most probably because the primary immune response to *M. tuberculosis* is cell-mediated, not humoral (2). Recent evidence suggests that the humoral response to *M. tuberculosis* is heterogeneous (3, 4). In one study of 59 patients with active tuberculosis, 52 (88%) had detectable antibody response to at least one of 10 mycobacterial antigens, but less than 50% responded to any single antigen (4).

Earlier serological tests used crude extracts such as tuberculin skin test material and had poor sensitivity and specificity (2). More recent tests, using highly purified antigens, have better sensitivity and specificity (2). The major advantage of these tests is that results are available within an hour and, because they involve simple technology, minimal equipment and little training are needed. In recent years, many manufacturers have marketed serological tests (5); these are readily available and relatively inexpensive (about US\$ 1 per test), which makes them attractive in resource-poor countries, where they may be aggressively marketed. However, the major disadvantages remain their poor sensitivity and specificity (5, 6). Sensitivity is highest in patients with smear-positive disease (3, 7, 8), but is much lower in children (9), patients with extrapulmonary disease (8, 10), HIV-infected individuals (11), and smear-negative cases (8, 10), i.e. the patients in whom another rapid test would be helpful because the AFB smear is insensitive. Specificity appears best in healthy volunteers in non-endemic countries (3, 7). It is much lower in appropriate test populations, such as close contacts of active cases, patients in whom active tuberculosis is suspected, and populations from endemic areas (6). Serological tests cannot reliably distinguish active tuberculosis from infection with *M. tuberculosis*. At present (2003), therefore, serological tests have no role in the diagnosis of tuberculosis.

Assays of cell-mediated immunity

In the past decade, molecular biological advances have spawned the development of tests to estimate cell-mediated immunity against *M. tuberculosis*. Circulating lymphocytes are extracted from samples of venous blood and exposed to purified antigens of *M. tuberculosis*; 6–24 hours later, the production of cytokines (inflammatory mediators, most commonly interferon gamma (12)) is measured.

The major theoretical advantage of this technique is that it measures the primary immune response of humans to tuberculosis. On the other hand, 20–47% of patients with extensive disease may be anergic at the time of diagnosis (13–16). Although temporary – resolving after a month or more of treatment (17) – this phenomenon could diminish the sensitivity and usefulness of this type of test, particularly in high-prevalence settings. A further disadvantage is that this is a highly complex test,

currently performed in only a few technically advanced research laboratories in industrialized countries. Further research to simplify and automate the technique, followed by validation work to estimate sensitivity, specificity, and predictive values, would be required before this test could be available for clinical use.

Amplification tests

Amplification tests, developed for many different microorganisms including *M. tuberculosis*, represent another application of molecular biological research to clinical practice. Highly specific nucleic acid probes (primers) recognize and attach to specific segments of the target DNA. The microorganism's DNA and the primer are replicated over many cycles so that the DNA is copied over and over – or “amplified”. Once amplification is complete, a DNA probe is added that binds only with the amplified DNA from the microorganism, producing a colorimetric reaction that can be measured (18).

The major advantages of this technique are that: results can be available in several hours (although some amplification tests take several days (19)); specificity can be 98–100% (20); sensitivity of these tests is greater than 95% in sputum that is AFB smear-positive, although only 50–60% in smear-negative, culture-positive specimens (21–23). Recently developed amplification tests may have better sensitivity in smear-negative specimens, while retaining the same high degree of specificity (19, 20, 24).

The major disadvantages are cost, complexity, and lower specificity (higher proportion of false-positives) under field conditions (25, 26). “In-house” tests may be cheaper but they take longer, are more difficult to perform (19), and require more highly trained technicians. Highly automated systems are available but initial capital costs and recurrent cost per test are both high, exceeding US\$ 15 per test. High costs and/or complexity currently make these tests inappropriate for application in high-prevalence, resource-poor settings. However, a test that costs as much as US\$ 6 may be cost-effective in such settings because patients with active disease are detected earlier and treatment is avoided in patients who are clinically suspect yet do not actually have active tuberculosis. The promise of amplification tests has often been limited by a high proportion of false-positive results obtained under programme conditions. Lack of sensitivity for sputum-negative patients and inability both to quantify mycobacteria and to distinguish viable from non-viable bacilli also limit the incremental benefits of this test, beyond the information provided by the AFB smear.

Summary

The AFB smear is inexpensive and highly specific, but it is labour-intensive and does not detect patients with smear-negative and extrapulmonary tuberculosis. Newer immunodiagnostic tests are the focus of active research, as they offer the promise of rapid and accurate diagnosis using equipment, materials, and personnel appropriate

for a resource-limited setting. At present, however, no serological test is available that can be recommended for routine clinical use, and tests of cell-mediated immune response to *M. tuberculosis* antigens are applicable only in research settings. Amplification tests are promising because they have good sensitivity and potential for good specificity and can be applied directly to clinical specimens such as sputum. At the moment, the high cost of equipment and materials and low specificity under field conditions make them inappropriate for resource-poor settings. None of the newer tests allows quantification of *M. tuberculosis* in sputum. Thus, even if a new, low-cost, simple method were to become available, the AFB smear might still be required to identify and monitor the most contagious and seriously ill patients.

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19. How can public and private sectors cooperate to detect, treat, and monitor tuberculosis cases?

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Accountability is a fundamental principle of tuberculosis control. In each geographical area, one individual (the District or Municipal Tuberculosis Control Officer) is responsible for the prompt detection, effective treatment, and systematic monitoring of tuberculosis cases. Poor treatment practices in any part of the health sector will increase the risk of drug resistance, spread of tuberculosis, and death. The tuberculosis officer must therefore be responsible for *every* tuberculosis patient in the jurisdiction, and not only for patients in the public health system.

In many countries, public health care institutions provide a decreasing proportion of health care services (1). Other providers include charitable organizations and health care services for government employees, insured workers, prisoners, and armed forces personnel and their families. In many countries, a significant proportion of patients with tuberculosis first consult private health care providers (2). These providers include licensed and unlicensed doctors and, in a large number of countries, trained and untrained pharmacists who sell tuberculosis drugs without prescription. Patients' use of these providers may reflect dissatisfaction with services offered by the public health care system. Unfortunately, care by private providers often results in delayed diagnosis, partial and non-standard treatment, drug resistance, spread of infection, and unnecessary expenditure by the patient.

There is no one perfect means to achieve coordination between public and private sectors in all countries. Effective programmes employ several, or all, of the approaches outlined below, but, whatever the approach, effective governmental services are a prerequisite for success.

Competition

To some extent, competition is a factor in nearly all tuberculosis control programmes: "Well-organized outpatient chemotherapy, especially if provided free of charge, will attract symptomatic cases from far and wide" (3). This approach can be effective if governmental services are free, convenient, patient-friendly, and reliably curative, and are widely recognized as such.

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Exclusion

Another approach is to exclude the private sector. All developed and some developing countries ban over-the-counter sales of anti-tuberculosis drugs, and in some countries these drugs are available only through the public sector. Only a few countries, however, control the medications that doctors can prescribe. Tuberculosis control programmes should try to prevent over-the-counter sales of anti-tuberculosis drugs, but any more ambitious form of exclusion requires political, cultural and social acceptance, as well as reliable tuberculosis control services within the public sector. These conditions exist in relatively few areas.

Contracting

The public sector can contract tuberculosis control services to private groups. The government does not have to provide all care; clinical services can be delegated to other health service providers. However, it remains the government's responsibility to ensure that effective clinical services are reliably available to patients. Clear expectations and clearly defined roles are essential for successful contracting.

Engagement

Some programmes actively engage the private sector in tuberculosis care. In many countries, public health programmes and professional groups, such as national chest societies, collaborate to establish standards of care that apply to both public and private sectors, and to revise medical school curricula to reflect these consensus standards. The New York City tuberculosis control programme illustrates this approach. In New York City, all doctors, including those in training, receive compact reference guides on diagnosis and treatment of tuberculosis, including information on where and how to refer tuberculosis patients. Standards of care for diagnosis and treatment are also disseminated widely through conferences, lectures, grand rounds, and circulars. High-quality laboratory services are provided to private patients free of charge; private laboratories and hospitals transport specimens to the Department of Health laboratory for testing. The Department of Health strongly encourages referral of patients to its chest clinics and urges doctors not to initiate treatment unless they can ensure its completion. The Department also provides observation of treatment by public health workers as a service to patients of private doctors, as long as the private doctors prescribe standard treatment regimens. Moreover, the Department provides free medications to patients of private doctors, provided that these medications are given in standard regimens and by direct observation. A "hotline" for physicians provides clinical consultation as well as patient-specific information.

There is often a long tradition of mutual disrespect between medical school providers and the public health system. This antagonism can be overcome only by sustained, concerted and technically sound efforts.

Reporting

Reporting, or notification, has been critical for effective tuberculosis control in many areas (4). Public health agencies that conduct active surveillance of laboratories, preferably with the authority to revoke laboratory licences for poor performance or failure to report cases, can greatly increase the reporting of smear-positive (and culture-positive) tuberculosis cases. The names of bacteriologically confirmed patients can be entered into a register and their treatment can be monitored. It is then possible to evaluate the outcome in every patient with bacteriologically confirmed tuberculosis from every institution in a reporting area. The approach taken should be supportive and collegial, and the process should create minimal disturbance for the laboratory. Laboratories can be sent regular updates about tuberculosis and about recent developments in the field. Laboratory directors can be involved in discussions about how to improve coordination, possibly through an advisory group representing directors of major laboratories. This approach can greatly increase detection rates, particularly when combined with efforts to educate doctors about the importance of acid-fast bacilli smears in diagnosis and with assured laboratory quality. It also focuses public health attention on bacteriologically positive cases, which account for most tuberculosis transmission and mortality. The system greatly facilitates surveillance, because there are many fewer laboratories than there are individual physicians.

As a minimum, public health programmes should maintain a list of all large providers of care, and should attempt to involve them in standardized diagnosis, treatment, and monitoring of tuberculosis. Tuberculosis control programmes relying on the public sector have been highly successful (5–9). As the level of government effectiveness increases, private facilities can be more effectively monitored, care standardized, and reporting ensured. Provided that private providers adhere to policies for care and reporting, public agencies can usefully support these providers. Thus, the role of the public sector should be not only to provide care but also to ensure high-quality care in all sectors – that is, to be accountable for all, or nearly all, patients in the area.

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