Present Position of Microscopy and of Culture in Diagnostic Mycobacteriology[1]

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Summary

The relative merits of microscopy and culture are discussed; it is attempted to review the possible reasons why microscopy is considered as unreliable in TB diagnosis, including the probably false application of Bayes' theorem. In the author's opinion, microscopy still holds a firm position in the rapid and relatively reliable detection of pulmonary TB. As for culture, it is highly questionable if "better media" could accelerate the growth rate of tubercle bacilli. Therefore, it should be attempted to introduce novel techniques which will allow to decrease considerably the mycobacterial mass needed for detection. Laboratories equipped with such devices could offer their services even on an intercontinental basis, in order to have a favourable cost-benefit ratio.

Zusammenfassung

Die relativen Vorteile der Mikroskopie und der Kultur werden besprochen; es wird versucht die Gründe zu analysieren, warum die Mikroskopie oft als nicht zuverlässig für die TB-Diagnostik betrachtet wird, einschließlich der vermutlich falschen Anwendung des Bayes'schen Theorems. Nach Ansicht des Verfassers stellt die Mikroskopie noch immer eine schnelle und verhältnismäßig zuverlässige Methode für die Diagnostik der Lungen-Tb dar. Was die Kultur anbelangt, es ist sehr unwahrscheinlich, daß „bessere" Nährböden imstande wären, die Wachstumsgeschwindigkeit der Tuberkuloseerreger zu beschleunigen. Man sollte deswegen anstreben, neuere Kulturverfahren einzuführen, die fähig wären, die für die Entdeckung notwendige Mykobakterienmasse beträchtlich herabzusetzen. Mit solchen Maschinen ausgerüstete Laboratorien könnten ihre Dienste sogar für andere Kontinente anbieten, um der Kosten-Nutzen-Analyse gerecht zu werden.

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This report covers exclusively pulmonary tuberculosis, because of its relative prevalence compared with that of the extrapulmonary disease.

Bacteriological investigation of sputum is a direct way of diagnosing tuberculosis, since the demonstration of tubercle bacilli is conclusive (27). Also, it was decided (26) that, from the epidemiological point of view, a "case" of pulmonary tuberculosis refers to a person with bacteriologically confirmed disease. Problems involved in this type of definition have been repeatedly reviewed (eg. 20).

In both industrialized and developing countries bacteriological diagnosis of tuberculosis presents many difficulties. In Africa (3) not much more that 30 % of the existing smear-positive cases are detected, diagnostic services were able to find between 10 % in Western Malaysia and 50 % in Japan (17), and a detection level of some 80 % is estimated in the Federal Republic of Germany (11).

In some industrialized countries, eg. in the USA, the reliability of microscopic examination was questioned (eg. 15), possibly also because of the incorrect application of the Bayes' theorem (21) which states — among others — that, as prevalence of a disease in the population decreases, a larger fraction of diagnostic tests will become falsely positive (8, 9). By constructing a fourfold correlation table (eg. 8, 24) it is possible to calculate the sensitivity (= its capacity to identify correctly those individuals who have the disease, or "true positives") and the specificity (= its capacity to correctly identify those who are free from the disease, or "true negatives") of a given diagnostic test. It is, however, true that a third parameter is important to assess the accuracy of test results: the predictive value (of positivity or negativity) can be defined as the probability of having (not having) the disease among the group of persons classified as positive (negative) by the test in question. It is easy to demonstrate (24) that, even with the sensitivity and specificity of 99 %, ie. a situation which cannot be achieved in the case of sputum smear investigation, assuming a prevalence of the disease around 1 %, only a predictive value of positivity of some 50 % would be encountered, whereas with a 10 % prevalence of the disease the predictive values would significantly exceed 90 %. So, the conclusion is that, whenever a *single* diagnostic test is used, its accuracy is bound to be poor if the frequency of the disease in the population examined is low. However, the usual procedure involves increasing the prevalence by preselection: investigation of respiratory symptomatics, of persons with X-ray abnormalities etc. So, though the Bayes' theorem is essentially correct, it applies *only* to situations where a single test would be applied to unselected general population, eg. indiscriminate mass case-finding by a single X-ray examination.

Analysing examples of the overall correlation of positive smear to culture results (Table 1), this ratio attains levels from 22 % to 62 %. There are basically two main reasons for such a fluctuation of results: the epidemiological and the technical one.

From the epidemiological point of view, several countries with more or less high tuberculosis prevalence have based their case finding on sputum smear examination of persons coughing for more than roughly 4 weeks (reviewed eg. in 22). If, however, the prevalence of tuberculosis is low and that of chronic cough high, which eg. is the case in the highlands of Papua-New Guinea (19), one needs to investigate some 1.400 "coughers" to detect a single tuberculosis case. On the other hand, approx. 7 % of the chronic coughers from the Papuan coast, or from Eastern Nigeria, or from Southern India will be positive on smear, needing effectively only some 14 "coughers" — or 100 times less than in the former case — to detect a single tuberculosis patient (19).

The technical factors include first an epidemiological survey situation as opposed to a case-finding programme (Table 2), furthermore the type (Table 3) and the number

Table 1. Overall smear/Culture positivity correlation

Reference/Country	Smear/Culture ratio
Boyd and Marr*/USA	22%
Marraro and coworkers**/USA	24%
Rickmann and Moyer***/USA	25%
Burdash and coworkers°/USA	43%
Pollock and Wieman°°/USA	50%
Kubica°°°/Africa, Europe,	53% Z–N
USA and Asia	63% Fluor.
Blair and coworkers #/USA	62%
Urbanczik ##/FRG	37%
Petersen ##/FRG	54%

*   Ann. int. Med. 82 (1975) 489
**   J. Amer. med. Technol. 37 (1975) 277
*** J. clin. Microbiol. 11 (1980) 618
°   J. clin. Microbiol. 4 (1976) 190
°°   J. clin. Microbiol. 5 (1977) 239
°°° Bull. int. Un. Tuberc. 55 (1980) 117
Amer. Rev. resp. Dis. 115 (1976) 427
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(Table 4) of sputum specimens recollected. As for the first, epidemiological surveys estimate the proportion of individuals suffering from the disease; they are carried out for the sake of knowledge, irrespective of whether action follows immediately, later or not at all. For case-finding purposes the individuals are sought who have the disease; it would be meaningless when proper chemotherapy would not be available for all patients discovered. This is why positive predictive values of about 70 % or even less

Table 2. Efficacy of smear and culture procedures (case finding)*

	Percentage of total cases found by procedure	
	A**	B**
Single sputum examined by microscopy only	40,6%	28,4%
Single sputum examined by microscopy and culture	57,8%	40,5%
Two specimens examined by microscopy only	45,3%	31,7%
Two specimens examined by microscopy and culture	71,9%	50,3%

*   K. D. Gautam, Indian J. Tuberc, 27 (1980) 24–32
** A = Survey depends basically in investigating symptomatic persons
    B = Survey depends basically in investigating the entire population over 5 years of age

Table 3. Positive results of sputum microscopy and culture related to the type of the specimen recollection*

Study	Type	Microscopy		Culture	
		Total	% posit.	Total	% posit.
1	overnight	160	85,0	160	98,1
	spot	160	51,8	160	91,8
2	overnight	181	31,5	179	51,6
	spot	179	13,9	179	42,2

1 Pande, Gautam, Mehrothra and Misra, Indian J. Tuberc. 21 (1974) 192
2 R. Valladares and R. Urbanczik, National Institute of TB, Caracas 1968–1969
* Specimens collected in the same persons, ZN technique was used in India, fluorescence in Venezuela, Petroff technique for culture in both.

could be acceptable for epidemiological surveys estimating morbidity, but certainly not for a case-finding programme. Considering the type of specimen, overnight sputum is obviously better than the sputum collected on the spot in the case of microscopy, much less so with culture (Table 3); for operational reasons, it will be often necessary to depend on one spot and one overnight sputum specimen. Thus, two specimens (Table 4) seem to detect most positives on smear – even more so on culture – while adding more specimens (Table 5) will not increase considerably the yield, particularly if a cost benefit relation is considered, though three specimens might be optimal if they can be afforded.

A very important problem is the definition of what is "positive" on smear – eg. more than 3 or more than 10 acid-fast rods per smear? This issue is thoroughly discussed in (10). Also, since all mycobacteria are acid-fast, it is of interest if (Ziehl-Neelsen method) differential identification of acid fast bacilli in sputum smears is possible: 4

Table 4. Positive on smear or in culture related to the number of specimens recollected*

Study	Smear (+)			Culture (+)			
	1st	2nd	3rd		1st	2nd	3rd sputum
1 (N = 191)	81%	14%	5%		92%	7%	< 1%
2 (N = 271)	80%	12%	8%		90%	9%	< 1%
3 (N = 1162)	86%	14%	–	M+	93%	7%	–
				M–	68%	32%	–
4 (N = 200)	82%	10%	8%	M+	95%	4%	< 1%
				M–	75%	23%	2%

* Only diagnostic cases included, ie. no treatment control
1 L. Herrera Malmsten, Chile (personal communication)
2 R. Urbanczik, Schömberg, 1979–1980
3 Chan and coworkers, Bull. Wld Hlth Org. 45 (1971) 551
4 R. Urbanczik, Schömberg, 1982–1983

readers examined an assortment of 200 smears, of which half were known to contain
M. tuberculosis and half were known to contain mycobacteria other than tubercle
bacilli. In two separate trials reader consistency ranged from around 70 % to around
86 %, and the overall accuracy attained 62 % to 80 %, which may roughly represent
the potential value of smear-based identification, perhaps exceeding the reliability of
tuberculin testing or radiology if experienced microscopists were involved (7).

Due to the low tuberculosis prevalence, to administrative complications and to a
general lack of interest in tuberculosis, eg. in the Federal Republic of Germany (16),
among all laboratories active also in the field of mycobacteriology 34 laboratories
investigated 5.000 smears or less in one year, which means roughly 100 or less in a
week; with the reported positivity rate of around 2 % it is easy to compute that these
people had a chance to see about 2 positive smears in a week.

Summarizing all this sputum smear examination may be very useful, even in indus-
trialized countries, provided it is understood as an important diagnostic tool in indi-
vidual patients and/or in specific groups of persons with allegedly high tuberculosis
prevalence (eg. drug addicts in general), and provided it is reasonably centralized to
keep the microscopist motivated and the cost per diagnosed case low.

Table 5. Number of sputum specimens required to establish positivity with smear in 270
patients*

Specimen sequence No.	Cumulative % patients with		Δ
	+ smear	+ culture	
1	47,4%	74,1	26,7%
2	53,0%	83,3	30,4%
3	58,2%	88,9	30,7%
4	59,3%	91,5	32,2%
5	60,4%	94,4	34,1%
6	61,9%	95,6	33,7%
7–17	67,0%	100,0	33,0%

* E. B. Blair and coworkers, Amer. Rev. resp. Dis. 113 (1976) 427–432

As for culture, its yield of positivity in relation to that of microscopy was shown in
Tables 4 and 5; it is of interest that, according to some investigators (22) the propor-
tion of those positive already on smear among all positives on smear and culture will
achieve in the course of a tuberculosis control programme a given value – eg. 40 % –
which will not continue to decrease, possibly due to the fact that even under "optimal"
conditions the case-finding will be predominantly passive, ie. symptomatics will search
relief by visiting medical facilities and not vice versa.

Unfortunately, it cannot be denied that culture technique did not change much from
Koch' or Petroff' times (18). It is highly questionable if "better" media may accelerate
the growth rate of tubercle bacilli: as reviewed recently (25), the M. tuberculosis DNA-
dependent RNA-polymerase exerts properties which result in a rate of RNA chain
growth only approximately one-tenth of that seen in Escherichia coli. Consequently,
novel procedures like detection and recovery of mycobacteria by radiometric techni-
ques (6, 13, 14) must be seriously considered since they allow to decrease considerably

the amount of mycobacterial mass susceptible to detection – it would no longer be necessary to wait for "colonies" seen by the naked eye. Also, it may well be that, in order to compensate partly for the quality of a few properly recollected sputum specimens, it would be feasible to introduce *sputum culture examination on a large scale* knowing that a part of such examination would be possibly useless. A similar situation already exists in clinical chemistry where automatic devices, eg. SMA, yield for each serum sample 20 or more biochemical parameters which might or might not be of importance to the clinician. By the same token, each sputum specimen received in a bacteriological laboratory might also be screened for the presence of mycobacteria. Since transport of specimens for mycobacterial investigations, even for intercontinental distances, presents today no serious problems at all (at least as far as technology is concerned), it is also possible to envisage a big laboratory in an industrialized country investigating specimens from a hospital in a developing country. In this way the problems with tuberculosis detection in many parts of the world might be resolved much more rapidly than waiting for the development of local laboratory networks.

Finally, it should be mentioned that with culture there are also problems involved in the definition of a "positive" specimen. The significance of isolating low numbers of colonies of *M. tuberculosis* in culture of sputum specimens was seriously questioned (1, 2, 4), and transfer from positive to negative specimens in the laboratory was, among others, suggested as one of the reasons (12), particularly in patients undergoing chemotherapy. Whereas no carrier state is recognized with *M. tuberculosis,* the question of colonization as opposed to invasion is a frequent problem with mycobacteria other than tubercle bacilli, as reviewed recently (5).

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