Source: P.D.O. Davies, 1994, Clinical Tuberculosis, Chapman & Hall Medical, London

# THE MICROBIOLOGY OF TUBERCULOSIS

3

*P.A. Jenkins*

## 3.1 INTRODUCTION

The mycobacteria that cause classical tuberculosis are

- *Mycobacterium tuberculosis*
- Asian variant
- African I
- African II
- *Mycobacterium bovis*

*M. tuberculosis* and *M. bovis* are probably the same species and are more accurately referred to as *M. tuberculosis* var. *hominis* and var. *bovinus* respectively. However it is more convenient to refer to them as above. *M. africanum*[1] and the Asian variants differ from the typical *M. tuberculosis* in minor cultural and biochemical ways only. These distinctions are of purely epidemiological interest and have no bearing on management or prognosis. In the UK, *M. bovis* disease is largely reactivation of infection acquired many years earlier[2]. There is normally little need for contact tracing and patients are rarely smear-positive on direct microscopy. However *M. bovis* is naturally resistant to pyrazinamide and this drug should not be used.

The unique character of the mycobacteria is that they are acid-fast. Once stained by an aniline dye such as carbol fuchsin they resist decolorization with acid and alcohol and are thus termed 'acid- and alcohol-fast bacilli' or AAFB. This is generally shortened to 'acid-fast bacilli' or AFB.

Acid-fastness is thought to be related to the uniquely thick cell wall, which is composed of an interlacing layer of lipids, peptidoglycans and arabinomannans. The aniline dye forms a complex with this layer and is held fast despite the action of the acid–alcohol. This allows the detection of AFB in specimens with a simple staining technique – the Ziehl–Neelsen (ZN) stain, which has been in use for over 60 years.

The uniquely thick cell wall also gives mycobacteria another useful characteristic, which is their resistance to the lethal effects of acids, alkalis and detergents. This is fortunate because most specimens are heavily contaminated with other bacteria, which grow more quickly than mycobacteria and would thus swamp the culture. It is therefore possible to 'treat' the specimen by adding an acid or alkali, thus killing the other bacteria leaving the mycobacteria alive. Ultimately the mycobacteria will also be killed and a balance has to be struck. Ideally between 2% and 3% of cultures should be lost because of contamination, indicating that the treatment procedure is neither too harsh nor too moderate.

The laboratory diagnosis of tuberculosis thus still relies on direct microscopy and culture using techniques devised many years ago. There is no 100% reliable serological test for tuberculosis (Chapter 17a, p. 367 *et seq.*). Many have been tried but all lack specificity

*Clinical Tuberculosis*. Edited by P.D.O. Davies. Published in 1994 by Chapman & Hall, London. ISBN 0 412 48630 X

and sensitivity. This is due to the ubiquitous nature of mycobacteria. They are widely distributed in the environment and exposure is unavoidable. As a result antibodies develop to the cross-reacting antigens that are present in most mycobacteria and this gives rise to false-positive reactions. On the other hand, individuals respond to different epitopes when infected and the use of a single antigen to detect antibodies gives false-negative results. Finally the widespread use of BCG vaccination may make interpretation difficult (Chapter 15d, p. 345 *et seq.*).

New approaches to the laboratory diagnosis of tuberculosis include the detection of specific substances that can only be present because of the concomitant presence of mycobacteria[3], the detection of mycobacterial antigens by an ELISA technique[4] and the polymerase chain reaction (Chapter 17b). However, microscopy and culture remain the primary methods of laboratory diagnosis and are likely to do so for many years to come.

Accurate and reliable identification and drug sensitivity tests are necessary for the successful treatment of tuberculosis and other mycobacterial diseases. The Public Health Laboratory Service (PHLS) has six laboratories designated as Regional Centres for Tuberculosis Bacteriology. These work in concert with the Mycobacterium Reference Unit to provide a service for England and Wales and currently process over 95% of all requests for identification and sensitivity tests. Standard techniques are used throughout the centres and sensitivity test results are subject to an on-going quality control system.

Most of the mycobacteria that cause disease in man are classed as category 3 pathogens and therefore require specialized handling in designated laboratories. Specimens suspected of containing mycobacteria and cultures thought to be mycobacteria therefore have to be processed in microbiological safety cabinets (class I or II) in a laboratory having a class 3 containment facility. This ensures that any aerosol that may be produced during the handling of the specimen or culture is unlikely to be a hazard to the laboratory worker[5].

## 3.2 SPECIMENS

Three specimens of sputum collected on successive days should be provided. Saliva is not adequate. A single specimen of sputum will miss about 25% of microspically positive and about 50% of culture-positive cases. If there is still a problem with diagnosis then an unlimited number of specimens should be sent.

When sputum is not available, for example in children, the gastric contents may be collected. Ideally the patient should cough and swallow for 10 minutes before collection, which should be done early in the morning before food is taken. Laryngeal swabs are less efficient than sputum or gastric contents.

Broncho-alveolar lavage using a fibre-optic bronchoscope provides excellent specimens from specific sites in the lung. However there is an increasing problem of contamination of such specimens because tap water is being used in the machines for rinsing the bronchoscopes instead of sterile distilled water. Unfortunately tap water contains environmental mycobacteria, which lodge in the plastic tubing that connects such machines to the mains supply. *M. chelonei* is the organism most likely to appear as a contaminant and it can be very difficult to eradicate[6]. However any one of the whole range of environmental mycobacteria can cause this problem, ranging from *M. avium* to *M. kansasii*.

Aspirates such as pleural fluids, CSFs etc. should be taken into sterile containers and transported to the laboratory as rapidly as possible. Actual pus is a better specimen than a pus swab but if possible a piece of tissue from the site should be sent. It is often the case that tissue is sent for histology and is placed directly into formalin, thus negating any chance of culture.

Genitourinary tuberculosis is relatively rare

in England and Wales and most laboratories lack the expertise to detect and interpret the significance of AFB in urine. However, when genitourinary tuberculosis is considered a possibility, then 50 ml of early morning urine should be sent on three successive days; 24 h specimens are not recommended.

The examination of faeces used to be rarely necessary. Most cases of tuberculous enteritis have lung lesions and tubercle bacilli in faeces come from swallowed sputum. However, with the advent of the acquired immunodeficiecy syndrome (AIDS) the situation has changed. A significant number of AIDS patients have disseminated disease due to *M. avium* and AFB are often demonstrable in both faeces and biopsies of the intestinal mucosa and can be cultured from these specimens.

### 3.3 MICROSCOPY

The patient whose sputum is positive on direct microscopy (Plate 1) is most likely to infect his/her close contacts[7]. Such a patient will have at least 5000 organisms/ml of sputum and may have up to ten times that number. Below 5000 organisms/ml it is unlikely that ZN or fluorescence staining will detect AFB. In England and Wales approximately 53% of new cases of pulmonary tuberculosis are positive on direct smear[8].

Direct microscopy of specimens other than sputum is of doubtful utility. Few laboratories have the expertise to interpret ZN-stained smears of urine and most no longer undertake this examination. Very careful scrutiny of smears from aspirates and tissues is necessary to detect the small number that are positive. These are most likely to be biopsy specimens from lymph nodes.

The main role of microscopy is therefore to identify the truly infectious patient so that the chain of infection can be halted by the appropriate treatment of the index patient. It is accepted that 2–3 weeks' chemotherapy with a modern antituberculosis regimen will make most patients non-infectious. Such a regimen will consist of isoniazid, rifampicin and pyrazinamide given for 2 months followed by isoniazid and rifampicin given for an additional 4 months[9] (Chapter 8b, p. 141).

### 3.4 CULTURE

The definitive diagnosis is the isolation of *M. tuberculosis* in pure culture. However this is only achieved in about 50% of cases and the diagnosis then has to rely on the clinical and/or radiological features, sometimes with histological evidence.

In the UK the most commonly used media for the isolation of tubercle bacilli are Lowenstein–Jensen (LJ) egg medium or Kirchner broth containing an antibiotic mixture. LJ is a simple egg-based medium that may be produced in a buffered form or as an acid egg medium[10]. Two tubes should be used, one containing glycerol and the other sodium pyruvate. Glycerol tends to inhibit the growth of *M. bovis* whereas sodium pyruvate encourages it.

From a sputum specimen positive by direct microscopy, LJ will show growth within 2–3 weeks at 37 °C. The colonies are rough and a beige-to-brown colour and show up well on the green background, which is due to the presence in the medium of the dye malachite green (Plate 2). Smear-negative sputa and specimens from other sites may not give a positive growth until they have been incubated for up to 6 or 8 weeks. Cultures showing no growth are reported negative at this time.

Kirchner being a liquid medium is more difficult to interpret and it is necessary to centrifuge the medium and make a ZN-stained smear of the deposit. If AFB are detected then subcultures are made on LJ but this inevitably entails a delay in reporting.

There are two other methods used in the UK for the culture of mycobacteria. These are the Roche MB check system and the BACTEC

460 radiometric system. Both these rely on the growth of the bacilli in a Middlebrook broth. This is a liquid medium that readily supports the growth of mycobacteria[11]. The Roche MB check system is a biphasic system in which the broth is inoculated with the specimen that has been decontaminated in the usual way. By inverting the system during incubation, this broth culture is flooded over a slope containing, on the one side, Middlebrook 7H-10 agar and, on the other side, a Middlebrook 7H-10 agar containing p-nitro-α-acetylamino-3-hydroxy propiophenone (NAP) and a chocolate agar. Mycobacteria growing in the broth will form visible colonies on the Middlebrook agar. If they do not belong to the tuberculosis complex they will also grow on the Middlebrook agar containing NAP. If they are not mycobacteria at all they will grow on the chocolate agar[12].

The BACTEC 460 radiometric system also uses a Middlebrook broth but one that incorporates a $C^{14}$-labelled substrate. During metabolism $C^{14}$-labelled $CO_2$ is produced and this is automatically monitored by the machine[11]. Using this system *M. tuberculosis* will grow, on average, 7–10 days more quickly than in the conventional LJ system.

The Middlebrook broth described above will not accommodate blood or bone marrow and is therefore of no use in disseminated disease. There is now available another medium designated Middlebrook 13A, which will take up to 5 ml of blood or a bone marrow. This is not so important from the point of view of classical tuberculosis but is very important in considering the problems of *M. avium* infections in patients with AIDS.

### 3.5 OTHER METHODS OF DIAGNOSIS

Despite considerable effort there is still no acceptable serological test for tuberculosis (Chapter 17a, p. 367). Such a test would be very useful for smear-negative pulmonary disease, extrapulmonary disease and tuberculosis in children. All the methods that have been tried have lacked specificity and sensitivity due to the diversity of the antigenic determinants of the tubercle bacillus and to exposure to environmental mycobacteria that share antigens with the tubercle bacillus.

ELISA tests have been described for the diagnosis of tuberculosis meningitis[13,14]. However, no ELISA system has yet proved to be acceptable in the UK. A murine monoclonal antibody to the 38 kDa antigen of *M. tuberculosis* has been used in a modified serological competition assay but unfortunately this is not generally available[15]. The application of the polymerase chain reaction for the direct detection of *M. tuberculosis*, while having enormous potential, is not in routine use at present[16–18] (Chapter 17b, p. 381).

Rather than look for antibodies, attempts have been made to detect either antigens themselves or substances specific for mycobacteria. Rabbit antisera to BCG have been used to detect antigens in CSF[19,20] and gas–liquid chromatography linked with mass spectrometry adapted for selected ion monitoring has been used to detect tuberculostearic acid (10-methyloctadecanoic acid) in both CSF and sputum[3,21]. Both techniques need further evaluation and the GLC–MS technique requires very expensive equipment.

### 3.6 IDENTIFICATION OF TUBERCLE BACILLI

It is possible to identify most mycobacteria to the species level with tests based on simple cultural and biochemical properties[22]. It is also possible to identify sub-specific variants by further biochemical tests, by phage-typing or by serotyping but this is of epidemiological value only and has little clinical relevance. It is thus important to identify a strain only as far as is clinically necessary. The recent

**Table 3.1** Cultural characters of tubercle bacilli after 2 weeks' incubation

| 25°C | 37°C | 45°C | PNB | THIA |
|---|---|---|---|---|
| | + | – | – | – |

PNB = p-nitro benzoic acid, THIA = thiacetazone } incubated at 37°C.

development of DNA fingerprinting using restriction fragment length polymorphism (RFLP) allows a very precise identification of a strain and its possible involvement in an outbreak situation (Chapter 17c, p. 391 *et seq.*).

Tubercle bacilli have a characteristic morphology when a ZN-stained smear is examined by microscopy. However it requires a considerable degree of expertise to interpret this and even then one can be misled. It is therefore necessary to set up a small number of cultural and biochemical tests to confirm the identity of a strain.

A suspension of the strain is inoculated onto LJ slopes for incubation at 25°C, 37°C and 45°C and also on to LJ slopes containing p-nitro-benzoic acid (PNB 500 μ2g/ml) and thiacetazone (10 μg/ml). These are incubated for 2–3 weeks and a tubercle bacillus will show the results illustrated in Table 3.1. Mycobacteria other than tubercle bacilli (MOTT bacilli), also called opportunist or non-tuberculous mycobacteria and even atypical mycobacteria, will show more rapid growth and/or growth at 25°C or 45°C and/or growth on the PNB slope and/or the thiacetazone slope. Some of them will also produce pigment either in the light only (photochromogen) such as *M. kansasii* or in both the light and dark (scotochromogens) such as *M. gordonae* (Chapter 13, p. 265).

Strains belonging to the tuberculosis complex can be divided into a number of variants as described in the introduction. The cultural characteristics for these variants are shown in Table 3.2 but, as said earlier, these are of little clinical importance except in the identification of *M. bovis*, which is naturally resistant to pyrazinamide. The inexpert administration of vaccine strain Bacillus Calmette–Guerin (BCG) can give rise to local abscesses and even regional lymphadenitis and it is not unusual to isolate BCG from such sites (Chapter 14b, p. 297). The characteristics of the strain have been included in Table 3.2 for convenience.

## 3.7 IDENTIFICATION OF OPPORTUNIST MYCOBACTERIA

The opportunist mycobacteria that most commonly cause pulmonary disease are:

- *M. kansasii*
- *M. avium*
- *M. intracellulare* } MAIS complex
- *M. scrofulaceum*
- *M. malmoense*
- *M. xenopi*

The identification of these organisms is best undertaken by specialist laboratories. The tests described for the identification of *M. tuberculosis* are supplemented by a test for the hydrolysis of Tween 80 and if necessary by thin-layer chromatography of an extract containing the superficial lipids from the cell wall[22,23]. Many species of mycobacteria have lipid patterns that are absolutely characteristic and not shared with other species. Sub-specific variants can also be identified using this technique.

Most if not all species of opportunist mycobacteria are widespread in the environment and they can gain access to specimens. It can therefore be difficult to decide which isolates are clinically significant. Multiple isolates in the absence of any other pathogen and with consistent clinical and/or radiological features will usually indicate significance.

## 3.8 DRUG RESISTANCE IN TUBERCLE BACILLI

Bacteria change their characters either: (i) in response to a stimulus – that is, by induced

**Table 3.2** Variants of the tuberculosis complex

| *Variant* | *Oxygen preference*[a] | *TCH*[b] | *Nitratase* | *Pyrazinamide* | *Cycloserine* |
|---|---|---|---|---|---|
| *M. tuberculosis* | A | R | + | S | S |
| Asian | A | S | + | S | S |
| African I | M | S | − | S | S |
| African II | M | S | + | S | S |
| *M. bovis* | M | S | − | R | S |
| BCG | A | S | − | R | R |

[a] A, Aerobic; M, microaerophilic; R, resistant; S, sensitive.
[b] Thiophen-2-carboxylic acid hydrazide, 5 mg/l.

adaptation; or (ii) spontaneously (genetically) by mutation. Mutations usually die out but if they have survival value they will multiply more than or instead of the original population of bacilli and will eventually replace it. All populations of tubercle bacilli generate small numbers that are resistant to any particular drug. If the patient is given the drug, a resistant mutant will not be affected and if it is not killed by the body's defences or another drug, it will multiply and replace the sensitive population. When a single drug is given the outcome is finely balanced – the defences may win over the very small initial number of mutants but they may fail. This is why single-drug therapy is often unsuccessful, resulting in drug resistance.

Excluding chemically related drugs, a mutant resistant to one drug is not especially likely to be resistant to a different drug. Thus, if two effective unrelated drugs are given together there is only a small chance of there being any mutant present that is resistant to both drugs at the same time. However, with very severe disease, immunity is low and the bacterial population is very large so doubly-resistant mutants may be present and there can be failure to eliminate them. This is why triple therapy is advisable initially – the chance of a triply-resistant mutant can be ignored. When the bacterial population is reduced (e.g. on conversion to culture-negative) it is safe to drop one of the drugs.

Examples of resistant-mutant incidence are $10^{-6}$ for rifampicin, $10^{-5}$ for streptomycin and isoniazid, $10^{-3}$ for cycloserine and $10^{-2}$ for pyrazinamide. Therefore the incidence of doubly-resistant mutants would be, for example $10^{-10}$ for streptomycin plus isoniazid.

## 3.9 SENSITIVITY TESTS TO TUBERCLE BACILLI

There are opposing views of the need for sensitivity tests. Most would agree that they are helpful in patients who relapse or do not convert and that if the technique is poor then they are not worth the effort. The disagreement arises over the advisability or need for sensitivity tests in new cases and the need to do tests for drugs other than streptomycin, isoniazid, rifampicin and ethambutol.

To plan treatment properly it is necessary to know the incidence of primary resistance in the country as a whole, and to determine this it is necessary to test a good sample of strains not just once but regularly. It seems reasonable therefore to carry out sensitivity tests on new cases if the facilities are available and the technique is adequate. Such tests also provide useful supporting evidence for the identity of the organisms.

There is a further disagreement on how sensitivity tests should be performed. It is simple to determine the minimum inhibitory concentration (MIC) of a drug. A control slope and others containing increasing con-

**Table 3.3** Example of a modal resistance control set

| | | *Drug concentrations* | | | | |
|---|---|---|---|---|---|---|
| | *Control* | *1* | *2* | *4* | *8* | *16* |
| Strain A | CG | CG | CG | – | – | – |
| B | IC | IC | + | – | – | – |
| C | CG | CG | IC | – | – | – |
| D | CG | CG | IC | + | – | – |
| E | IC | IC | – | – | – | – |
| Mode | | CG | IC | – | – | – |

CG, confluent growth; IC, innumerable discrete colonies; +, 20–100 colonies; –, less than 20 colonies.

centrations of the drug are inoculated equally and incubated. However, the results differ considerably in different laboratories owing to variations in medium or technique and to some extent at different times in any one laboratory. Even if the MIC is accurate – what does it mean? The MIC can be related to concentrations of drug attainable in the blood or tissues or lesions but these concentrations are not known for all drugs in all situations. Also, in the body the concentrations are always changing – furthermore there are sometimes antagonistic substances present and variations in pH or oxygen tension and other conditions that effect the potency of each drug in an unpredictable way. The MIC is an *in vitro* test under special *in vitro* conditions and there cannot be exact quantitative equivalence (i.e. in μg/ml).

The only valid method of assessing sensitivity tests is to relate the laboratory findings to the results of treatment, initially in animal experiments but then, more importantly, in clinical trials. Such an approach can establish the general outline of sensitivity technique and interpretation but it has only been used in man for a few drugs and then not thoroughly. The chief difficulty is that drugs are not used singly in therapy trials.

The problem has therefore been approached as follows:

1. Establish the behaviour of normal (wild) strains of TB.
2. Determine the response of the patient's strain.
3. Assume that any difference (loss) of sensitivity is harmful.

The normal disc-diffusion methods used to determine the sensitivity of most bacteria are not suitable for mycobacteria. The long incubation period means that plates dry out and the dynamics of the diffusion of the drug into the agar become very complicated. It is necessary therefore to test strains by a titration method. Tubes of medium containing a range of concentrations of the drug (usually doubling) are inoculated and incubated. The results can be expressed in three ways:

1. MIC – the minimal inhibitory concentration.
2. RR – the resistance ratio to a standard strain, usually H37Rv, (i.e. ratio of MICs).
3. MR – modal resistance; this compares the MIC of the test strain with the mode or most common MIC of a group of normal strains.

In the UK the modal resistance method is most commonly used[24]. The mode is determined by testing a large number of strains from new (untreated) cases of tuberculosis. In the example shown in Table 3.3 only five strains are used, but in practice five strains are tested every week, i.e. 260 a year.

### 3.10 PYRAZINAMIDE

Normal methods cannot be used as the drug is only active in acid medium (pH 5.0 to 5.4) and strains vary in their ability to grow at such low pH levels. It is best to test at three different pH levels, e.g. 5.0, 5.2 and 5.4. Vigorous strains give a true result a pH 5.0, feeble strains at 5.4 and others are intermediate. To reduce the work, each test can be limited to a control and a single drug tube.

### 3.11 CONCLUSION

The decline in the incidence of tuberculosis has seen a concomitant decline in expertise in general microbiology laboratories. Direct microscopy and culture of specimens is still undertaken in most hospital laboratories but as the number of positives has fallen there is evidence that lack of experience has led to false-positive results being issued. 'Pseudo' outbreaks have aroused unnecessary alarm [25]. False-negative results are much more difficult to identify but it is reasonable to assume that they occur. It is to be hoped that the increased incidence in tuberculosis arising from the problems in the USA and particularly that of multi-drug resistant strains will rekindle interest at all levels in a disease that was well on its way to being eradicated in most developed countries.

### REFERENCES


1. Castets, M., Rist, N. and Boisvert, H. (1969) La Variété africaine du bacille tuberculeux humain. *Méd. Afr. Noire*; **16**, 321–2.
2. Hardy, R.M. and Watson, J. (1992) *Mycobacterium bovis* in England and Wales: past present and future. *Epidemiol. Infect.*, **109**, 23–33.
3. French, G.L., Teoh, R., Chan, C.Y. *et al.* (1987) Diagnosis of tuberculous meningitis by detection of tuberculostearic acid in cerebrospinal fluid. *Lancet*, **ii**, 117–19.
4. Watt, G., Zaraspe, G., Bautista, S. and Laughlin, L. (1988) Rapid diagnosis of tuberculous meningitis by using an enzyme-linked immunosorbent assay to detect mycobacterial antigen and antibody in cerebrospinal fluid. *Infect. Dis.*, **158**, 681–6.
5. DHSS (1978) *A Code of Practice for the Prevention of Infection in Clincal Laboratories*, Department of Health and Social Security, HMSO, London.
6. Nye, K., Chadha, D.K., Hodgkin, P. *et al.* (1990) *Mycobacterium chelonei* isolated from broncho-alveolar lavage fluid and its practical implications. *J. Hos. Infect.*, **16**, 257–61.
7. British Thoracic and Tuberculosis Association (1978) A study of standardized contact procedure in tuberculosis. *Tubercle*, **59**, 245–59.
8. Medical Research Council Cardiothoracic Epidemiology Group (1992) National Survey of notification of tuberculosis in England and Wales. *Thorax*, **47**, 770–5.
9. British Thoracic Society (1984) A controlled trial of 6 months chemotherapy in pulmonary tuberculosis. Final report: Results during the 36 months after the end of chemotherapy and beyond. *Br. J. Dis. Chest*, **78**, 330–6.
10. Zaher, F. and Marks, J. (1977) Methods and medium for the culture of tubercle bacilli. *Tubercle*, **58**, 143–5.
11. Middlebrook, G., Reggiardo, A. and Tigertt, W.D. (1977) Automatable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. Am. *Rev. Respir. Dis.* **115**, 1066–9.
12. Damato, R S., Isenberg, H.D., Hochstein, L. *et al.* (1991) Evaluation of the Roche Septi Check AFB system for the recovery of mycobacteria. *J. Clin. Microbiol*, **29**, 2906–8.
13. Grange, J.M., Gibson, J., Nassau, E. and Kardijito, T. (1980) Enzyme-linked immunosorbent assay (ELISA): a study of antibodies to *Mycobacterium tuberculosis* in the IgG, IgA and IgM classes in tuberculosis and sarcoidosis. *Tubercle*, **61**, 145–52.
14. Sada, E.D., Ferguson, L.E. and Daniel, T.M. (1990) An ELISA for the serodiagnosis of tuberculosis using a 30 000 Da native antigen of *Mycobacterium tuberculosis*. *J. Infect. Dis.*, **162**, 928–31.
15. Wilkins, E.G.L. and Ivanyi, J. (1990) Potential value of serology for diagnosis of extra pulmonary tuberculosis. *Lancet*, **336**, 641–4.
16. Brisson-Noel, A., Gicquel, B., Lecossier D *et al.* (1989) Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. *Lancet*, **ii**, 1069–71.
17. Sjobring, U., Meckleburg, M., Bengard, A.A.

and Miorner, H. (1990) Polymerase chain reaction for detection of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.*, **28**, 2200–4.

18. Shankar, P., Manjunath, N., Mohan, K.K. *et al.* (1991) Rapid diagnosis of tuberculosis meningitis by polymerase chain reaction. *Lancet*, **337**, 5–7.
19. Sada, E., Ruiz-Palacios, G.M., Lopez-Vidal, Y. and de Leon, S. (1983) Detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay. *Lancet*, ii, 651–2.
20. Radhakrishan, V.V., Sehgal, S. and Mathai, A. (1990) Correlation between culture of *Mycobacterium tuberculosis* and detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis. *J. Med. Microbiol.*, **33**, 223–6.
21. Oldham, G., Larsson, L. and Mardh, P. (1979) Demonstration of tuberculostearic acid in sputum from patients with pulmonary tuberculosis by selected ion monitoring. *J. Clin. Invest.*, **63**, 813–19.
22. Marks, J. (1976) A system for the examination of tubercle bacilli and other mycobacteria. *Tubercle*, **57**, 207–52.
23. Marks, J. and Jenkins, P.A (1971) Thin layer chromatography of mycobacterial lipids as an aid to classification: technical improvements: *Mycobacterium avium*, *M. intracellulare* (Battey bacilli). *Tubercle*, **52**, 219–25.
24. Marks, J. (1961) The design of sensitivity tests of tubercle bacilli. *Tubercle*, **42**, 314–16.
25. Davies, P.D.O. Williams, C.S.D., Shears, P. *et al.* (1992) Pseudo-outbreak of tuberculosis in a paediatric oncology ward [Abstract]. *Thorax*, **47**, 220 P.