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The New Diagnostic Mycobacteriology Laboratory

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Recent surveys in the USA show that many mycobacteriology laboratories continue to use less-than-optimum culture and susceptibility testing methods. This seems to be true for European countries as well. The past few years have brought significant changes to the clinical tuberculosis laboratory. High-performance liquid chromatography and direct detection of acid-fast bacilli in clinical specimens aim at the same goal: increased sensitivity and specificity of the diagnostic approach and reduction of turnaround time. This review outlines a brief comparison between contemporary traditional methods and the latest developments in the direct detection of acid-fast bacilli. If patient care and public health are always considered paramount, regardless of admission time, hospital type, etc., the current concept of services has several shortcomings. One way to manage this situation is to sort and allocate specimens according to a system of priorities. There is a growing realization that no single method by itself is the best. To streamline the best choice for laboratory diagnosis, an additional *dynamic acid-fast network* is presented: 'Point-of-Care,' 'Fast Track,' and 'Specialty' laboratories. The physician interacts with all three types of laboratories, so ongoing communication between the physician and the laboratory is essential. Laboratorians must work together in the formation of this *dynamic acid-fast network* to improve service rendered for our patients.

One of the most alarming aspects of the recent increase in tuberculosis in certain areas of the world has been the occurrence of nosocomial outbreaks with multidrug-resistant strains, especially in facilities providing health care for HIV-positive individuals. As demonstrated recently, tuberculosis infection in HIV-infected patients spreads readily and progresses rapidly to active disease (1). The new recommendations by the Centers for Disease Control and Prevention/American Thoracic Society (2) require in vitro drug susceptibility testing of *Mycobacterium tuberculosis* isolates from all patients and reporting of these results to the local health department. It has also been recommended that patients receive four-drug therapy until results of susceptibility tests are known. This puts the clinical laboratory under additional time pressure. The message for the *new* diagnostic mycobacteriology laboratory is thus evident: *shorter turnaround times.*

In 1958 Middlebrook and Cohn (3) stated that "there is much evidence that the clinical-bacteriologic investigation of tuberculosis in this country (USA) today is pitifully inadequate." Ellner and Elbogen (4) noted more than 25 years ago in their paper entitled "Modern Methods in Tuberculosis Bacteriology for the General Hospital": "It has become apparent that there is a considerable hiatus, at the informational level, between the methods used in tuberculosis bacteriology by specialized research and reference laboratories and those used by smaller general laboratories." And today? How new is the "new diagnostic laboratory"? The Centers for Disease Control and Prevention (5) surveyed 56 state and territorial public health laboratories, only to find a minority of laboratories that were using rapid methods for identification and susceptibility testing. Surprisingly, a questionnaire completed by 1,517 participants in the College of American Pathologists Mycobacteriology E survey revealed similar data (6). Only 26 % of the respondents processed respiratory specimens daily and provided microscopy results within 24 hours after the specimen had been received.

The laboratory provides an important service to the physician in diagnosing mycobacterial dis-

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eases and in monitoring therapy. However, the laboratory is not at the beginning, rather it is almost at the end of the decision tree for the patient's health improvement: first, because the symptomatic patient may wait several weeks until he seeks help at the doctor's office or, worse, is evaluated at an emergency room; second, because the physician may or may not initially recognize mycobacterial disease; and third, because an unsatisfactory specimen may have been sent to the mycobacteriology laboratory. Unfortunately, it is exactly at this point where miracles are expected from the microbiology laboratory.

Laboratories that provide clinical microbiological services may exist in physicians' offices, clinics, complex medical centers, general and specialty hospitals of all sizes, local and regional reference laboratories, and local, state and federal public health laboratories. With such a variety of laboratory types, there is no space for the philosophy of "one size fits all": every laboratory is different. However, they all have the same task of providing accurate results in a timely manner.

Let's briefly entertain five clinical scenarios. First, with highly infectious pulmonary tuberculosis with acid-fast smear-positive sputum, the patient should be under adequate anti-tuberculosis treatment and in respiratory isolation as rapidly as possible to stop transmission (2); second, with tuberculous meningitis, the significance of the disease is disproportionate to its rarity, owing to the appreciable risk of irreversible neurologic damage if it is not promptly recognized and treatment initiated (7, 8); third, with unilateral cervical lymphadenitis in children less than 5 years old, the disease is usually caused by *Mycobacterium avium* complex or other nontuberculous mycobacteria which, aside from surgical excision, require no antimycobacterial therapy (9–11); fourth, with HIV-positive individuals, who are more susceptible to opportunistic infection than are immunocompetent patients, treatment is based on rapid identification of the organism (2, 12–14); and fifth, with intravesical instillation of *Mycobacterium bovis* [BCG] as the treatment of choice for superficial, transitional cell bladder cancer, dissemination and subsequent detection of *Mycobacterium bovis* [BCG] from the respiratory tract is possible and should not be misidentified as *Mycobacterium tuberculosis* (15–17). The physician must be assured that in all of these situations the specimens are handled efficiently in the mycobacteriology laboratory, providing accurate results in a timely manner. How should the laboratory carry out this responsibility?

The few past years have brought significant changes to the clinical tuberculosis laboratory. Direct detection of mycobacterial compounds by highly sophisticated chemical methods such as gas-liquid chromatography/mass spectrometry (GLC/MS) (18) or direct detection of acid-fast bacilli (AFB) in clinical specimens by molecular biological methods (19, 20) are essentially aiming at the same goal: increasing sensitivity and specificity of the diagnostic approach on the one hand, and reducing turnaround time on the other. In the following, a brief comparison between contemporary traditional methods (all of which are growth-dependent) and the latest developments in the direct detection of AFB will be given.

I. Detection and Identification of Mycobacteria from Clinical Specimens

Ia. Traditional Methodology

Microscopy

Despite the many recent advances in mycobacteriology, early laboratory diagnosis of tuberculosis still relies heavily upon the examination of stained smears (Figure 1). Not only is it still the easiest, cheapest and most rapid procedure, but it also provides the physician with important preliminary information. For example, in as many as 95 % of the microscopically positive cases in HIV-negative patients, tuberculosis is the causative agent of the disease (21), except for southern parts of the USA, where the positive predictive value for tuberculosis is less than 60 %, especially in noncavitary cases (47 %) (22). The sensitivity of the AFB smear is, however, considerably lower than that of the cultural approach: the minimal number of AFB necessary to produce a positive direct smear has been estimated by several authors as approximately 5×10^4 per ml of sputum (23). Depending largely on the extent of the lesion(s), the overall sensitivity for microscopy is only between 22 and 65 % (24–26). As shown by Urbanczik (24), examination of two smears will, however, detect most smear-positive cases, while testing more samples of the same kind will not materially improve the result. Upon using a cytocentrifuge for patients' sputum smears (n = 120) the correlation between positive AFB smears and positive AFB cultures increased to 100 % (27). Unfortunately, in this particular study, only solid media were used.

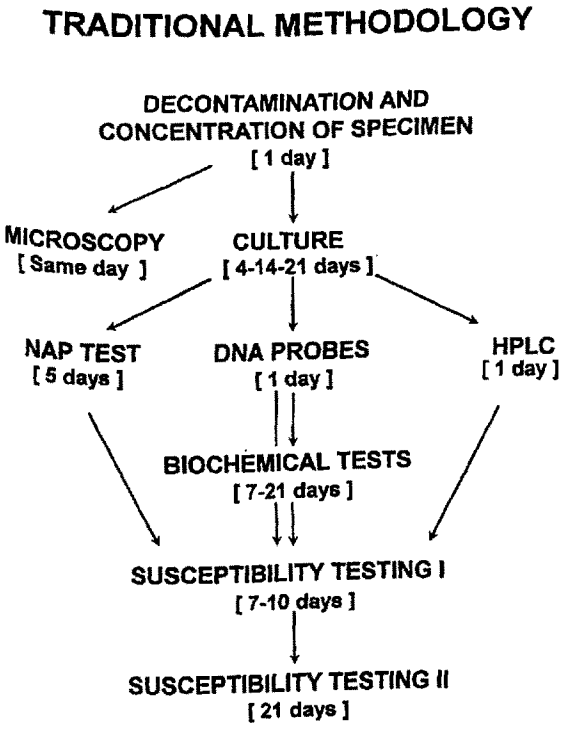

Figure 1: Laboratory diagnosis of mycobacteria (algorithm). Traditional methodology encompasses gentle decontamination and efficient concentration of the specimen, the use of solid and liquid media (radiometric detection is currently the fastest), combined with DNA probes when acid-fast bacilli are present. The Bactec system allows rapid screening for drug resistance (streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide). Conventional agar techniques can be used to obtain additional susceptibility data for isolates resistant to one or more of the first-line drugs. Appropriate average times are listed in brackets.

Staining the smears with a fluorochrome (e.g., auramine-rhodamine) is superior to the classical carbol fuchsin stain [Ziehl-Neelsen or Kinyoun (28–30)] since, with fluorescence microscopy, a lower magnification can be used, which both increases sensitivity and reduces the time for screening. It is good laboratory practice to confirm any smear-positive result (including those considered doubtful) in newly diagnosed patients by a Ziehl-Neelsen stain (31) or, alternatively, to have the smear(s) checked by a second person.

Culture

Pretreatment. Compared with other types of bacteria, which generally reproduce within minutes, mycobacteria proliferate extremely slowly (generation time 18 to 24 h). If specimens from nonsterile body sites are not decontaminated, mycobacteria will easily be overgrown by more rapidly dividing organisms, e.g., bacteria and fungi. The best yield of AFB may be expected when using the mildest digestion/decontamination method that manages to eradicate contaminants without interfering with the viability of the mycobacteria. Fortunately, AFB are quite refractory to harsh chemicals. In the past, a number of different digestion/decontamination procedures have been applied successfully.

In contrast to the USA, where specimens are mainly pretreated with N-acetyl-L-cysteine (NALC)/NaOH (according to the recommendations of the Centers for Disease Control and Prevention) (30), European laboratories frequently use sodium dodecyl (lauryl) sulfate (SDS)/NaOH (32). A study encompassing a total of 1,500 clinical specimens from the respiratory tract has revealed that (i) specimens treated with SDS/NaOH yielded more mycobacteria and fewer contaminants than specimens processed with NALC/NaOH ($p < 0.05$), and (ii), the SDS method is quite compatible with the radiometric detection system (Bactec) (33). Furthermore, SDS has also been applied prior to direct detection of *Mycobacterium tuberculosis* complex by a target-amplified test system (34). Other decontamination agents, e.g., Zephiran-trisodium phosphate (Z-TSP), also selectively destroy many contaminants while exhibiting little bactericidal activity against tubercle bacilli. However, Z-TSP appears to be more damaging to nontuberculous mycobacteria than other agents (35).

Media and Detection Time. Although it is generally accepted that mycobacteria grow more rapidly in liquid medium, it has been rarely, though repeatedly, observed that some of the mycobacterial isolates appear exclusively on solid media. Therefore, solid media should never be omitted. The current gold standard (36) consists of a combination of different culture media (nonselective/selective) that allow optimum culture sensitivity, i.e., an agar-based (e.g., Middlebrook 7H10/sel 7H11), an egg-based (e.g., Lowenstein-Jensen [LJ]), and a liquid medium (e.g., Dubos broth, Septi-Chek, Bactec 12B). Since its introduction, the Bactec radiometric system has considerably improved culture methodology. On average, it yields results one week earlier than Septi-Chek (37–39). For smear-positive specimens, Roberts et al. (40) have found for *Mycobacterium tuberculosis*, an average detection time of 8 days in the Bactec system and 19.4 days

in nonradioactive conventional media; for smear-negative specimens, Morgan et al. (41) reported an average recovery time of *Mycobacterium tuberculosis* between 13.7 days in the Bactec system and 26.3 days in conventional media. In addition to more rapid growth, Bactec technology offers the possibility of a preliminary screening test (*Mycobacterium tuberculosis* complex versus nontuberculous mycobacteria; see "NAP Test" below) and allows radiometric susceptibility testing for the five first-line antituberculosis drugs within a few days (Figure 1).

General Considerations for Special Specimens. (*a*) *Blood.* Blood specimens from immunocompromised patients can be processed in three ways: (i) in the Isolator system (Wampole Laboratories, USA); (ii) in the Bactec system (13A medium, Becton Dickinson Diagnostic Instrument Systems, USA); and (iii) by lysis with sodium deoxycholate. Recovery rates among these three techniques are approximately the same (42–44). Since *Mycobacterium avium* complex is known to be able to survive in Isolator tubes for at least one week at densities encountered in disseminated human infection, Isolator tubes are especially suitable for mailing blood cultures. Because growth may even occur in the Isolator tube, such blood cultures should, however, be processed within a few hours after sampling if a quantitative result is desired (45). On the basis of a recent study, the combined use of Isolator and radiometric 12B vials for recovery of mycobacteria is contraindicated (46) unless blood sediment prior to inoculating the 12B medium is carefully washed to eliminate any growth inhibitors. The flexibility to choose different culture media is a real advantage for those laboratories requiring versatility, although processing Isolator tubes is somewhat cumbersome.

In contrast, Bactec 13A vials do not require extensive manipulation of blood in the laboratory. Furthermore, Bactec vials have the advantage of permitting bedside media inoculation. On the other hand, it is well known that antituberculous therapy significantly prolongs the detection times of nontuberculous mycobacteria in Bactec 13A cultures (47), and drug carryover may even be responsible for false-negative cultures.

The minimum number of blood cultures required to reliably detect infection remains controversial (48, 49). A most recent study encompassing more than 1,000 blood cultures (50) has shown that the practice of routinely processing paired blood cultures should be discontinued, since specimens for repeat testing can readily be collected if the initial specimen remains negative after one or two weeks and disseminated *Mycobacterium avium* complex infection is still clinically suspected. The authors have, furthermore, demonstrated that acid-fast smears of sediments are definitely not a reliable means of detecting mycobacteremia.

(*b*) *Cerebrospinal Fluid.* The urgency of the clinical situation of patients with tuberculous meningitis demands immediate treatment, i.e. without waiting for culture results (8, 51). Too often, lumbar puncture must be performed with little forethought. Despite these particular circumstances, the physician must ensure that enough cerebrospinal fluid is promptly delivered to the laboratories for cell counts, chemistry, smears, and cultures (52). Several studies (53, 54) have recorded the overuse of cerebrospinal fluid cultures. To prevent such overuse, Albright et al. (55) showed that mandatory screening substantially decreased the culture rate. To establish the diagnosis of tuberculous meningitis, at least five cerebrospinal fluid specimens (preferably a total of 10–15 ml) should be submitted for culture. This is supported by the data of Kennedy and Fallon (56) who found an increase in cumulative smear-positivity, i.e., from 37 % on the initial sample to 87 % by the fourth specimen, when multiple specimens of smaller volumes (e.g. 3 ml) were provided.

(*c*) *Gastric Lavage.* A recent study reaffirmed that gastric suction provides the specimen of choice for the diagnosis of primary pulmonary tuberculosis in children (57). *Mycobacterium tuberculosis* could be cultured from gastric specimens of 10 of 20 children, whereas in only two cases the bronchoalveolar lavage fluid (BAL) specimen was positive as well. Although the number of patients was small, this study demonstrated that in children, gastric lavage fluid obtained on three consecutive days represents by far the better clinical specimen than BAL in diagnosing smear-negative primary pulmonary tuberculosis.

Smear-Positive, Culture-Negative Specimens. A distinction must be made between specimens with a false-positive smear result and those specimens that are microscopically positive but whose AFB fail to grow (25). Independent of the clinical specimen, explanations for false-negative cultures include (i) nontuberculous mycobacteria that require special incubation conditions (e.g., temperature: *Mycobacterium marinum*, *Mycobacterium ulcerans*; supplemented medium: *Mycobacterium haemophilum*, *Mycobacterium paratuberculosis*); (ii) highly fastidious organisms

(*Mycobacterium genavense*); or (iii) mycobacteria isolated from patients under long-term antituberculous therapy.

Identification

Morphology of Mycobacteria. Apart from the morphology of the colonies on solid agar, little can generally be concluded from the microscopical appearance of the organisms. Although *Mycobacterium avium* complex usually tends to be coccobacillary and *Mycobacterium kansasii* forms rather elongated, thick, often beaded rods, a presumptive species diagnosis should never be attempted from microscopy slides. Some authors have found cord formation to be a sensitive indicator for *Mycobacterium tuberculosis* (58). The matter remains, however, controversial, since mycobacteria other than tubercle bacilli can also produce serpentine cords in liquid media [e.g., *Mycobacterium avium* complex, *Mycobacterium gordonae*, *Mycobacterium chelonae*, *Mycobacterium marinum* (59)]. At best, the observation of cords in broth may help determine which method to use to identify the isolate in the most rapid manner.

Nonisotopic Nucleic-Acid Probes. Culture confirmation tests using DNA probes (e.g., AccuProbe Culture Confirmation Test, Gen-Probe, USA) are now widely established in clinical mycobacteriology laboratories. The commercially available nucleic-acid probe assays (available at present: *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium avium* complex, *Mycobacterium gordonae*, *Mycobacterium kansasii*, *Mycobacterium tuberculosis* complex) are very easy to perform and dramatically shorten the turnaround time of the result inasmuch as species identification is obtained within a few hours (Figure 1). In addition, they can also be performed with broth from radiometric 12B vials (60). For *Mycobacterium tuberculosis* complex, the Accu Probe system has revealed an accuracy of nearly 100 % (60, 61).

Because of the increased detection of *Mycobacterium bovis* in immigrants (62) and because of an increasing number of patients with superficial bladder cancer undergoing treatment with *Mycobacterium bovis* [BCG] with the potential of dissemination (15), the AccuProbe result for *Mycobacterium tuberculosis* complex should not be considered as final identification. Speciation/identification of the species of these isolates helps to alert public health officials and to ensure proper patient management.

Blood causes a strong positive interference with the acridinium-ester-labeled probes. Two different approaches have been suggested to overcome the potential of false-positive results: first, to use a specimen blank (60), or second, for 13A Bactec vials, to subculture first into a 12B vial and incubate overnight prior to probing the cells (63). In very rare instances the *Mycobacterium tuberculosis* complex probe has reacted with strains of *Mycobacterium terrae* (64) or *Mycobacterium celatum* (65). Stockman et al. (66) have found eight isolates of mycobacteria that gave erroneous *Mycobacterium tuberculosis* AccuProbe results. DNA sequencing of some of the isolates resembling phenotypically *Mycobacterium avium* complex revealed that they were identical to a recently described distinct group of slow-growing mycobacteria (67). These false-positive results could be eliminated by extending the time of the selection reagent step from 5 min to 10 min in the assay (66).

In view of the increasing number of immunosuppressed patients, one should be aware that mixed infections may occur as well (68), i.e., that the specimen to be tested may contain nontuberculous mycobacteria in addition to *Mycobacterium tuberculosis* complex (69). Subculturing the positive broth cultures on solid media as well is, therefore, an absolute necessity.

NAP Test. *p*-Nitro-α-acetylamino-β-hydroxypropiophenone (NAP) is a precursor in the synthesis of chloramphenicol and specifically inhibits members of the *Mycobacterium tuberculosis* complex, while nontuberculous mycobacteria are not affected (70). This test, which can be performed radiometrically (Bactec), leads to a preliminary discrimination between *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria within five days (Figure 1). The NAP result must be considered preliminary, since the test yields a false result in 2 to 3 % (71).

Chromatographic Procedures. Qualitative and quantitative differences in the spectrum of mycolic acids present in the cell wall are a reliable criterion for identifying mycobacterial species. Once mycobacterial cultures are available, high-performance liquid chromatography (HPLC) offers rapid and easy identification of AFB (72–75) (Figure 1). Thibert and Lapierre (76) have identified 96.1 % of more than 1,100 strains (whereas the more time-consuming biochemical procedures together with commercial DNA

probes identified 98.3 %). HPLC also allowed early detection of rare mycobacterial species such as *Mycobacterium haemophilum, Mycobacterium malmoense, Mycobacterium shimoidei* and *Mycobacterium fallax* as well as uncharacteristic strains of *Mycobacterium simiae.* In addition, HPLC can be used to separate *Mycobacterium bovis* [BCG] from *Mycobacterium tuberculosis* and *Mycobacterium bovis* (77).

Mycobacteria can also be identified on the basis of cellular fatty acids by gas liquid chromatography (GLC) (78). If identification to the species level is not possible, a group consisting of a few species is usually obtained, which can then be further characterized by morphological and/or biochemical criteria. Most experience has been gained with the commercial Microbial Identification System (Hewlett Packard, USA/Microbial ID, USA). Once cells are extracted and derivatized, the GLC analysis itself is quite accurate and rapid (< 2 h). The need for standardized subcultures on Middlebrook agar is, however, a significant disadvantage. Additionally, the system's data base needs improvement (79). The advantage, however, is versatility for the clinical microbiology laboratory, because there are several data bases available that enable identification of groups of microorganisms other than mycobacteria.

Biochemical Tests. For many decades the standard biochemical tests (29, 30, 36) remained the only means of identifying mycobacterial species. Unfortunately, biochemical testing requires an additional two to four weeks for completion after mycobacteria have appeared in culture (Figure 1). Despite much exciting technological progress in the direct detection of AFB in clinical specimens (see "Direct Detection of Acid-Fast Bacilli in Clinical Specimens by Amplification," below), these tests cannot, at present, be eliminated from the clinical mycobacteriology laboratory. They are still required in cases where (i) species-specific DNA probes are not available; (ii) results obtained with other identification methods are equivocal or not interpretable; and (iii) discrimination within the *Mycobacterium tuberculosis* complex is necessary (see below).

Identification of Members within the Mycobacterium tuberculosis Complex. Intravesical therapy with *Mycobacterium bovis* [BCG] has proved to be more effective in the prophylaxis and treatment of superficial bladder tumors and carcinoma in situ than most therapeutic agents. This superiority of *Mycobacterium bovis* [BCG] immunotherapy has increased its use throughout the world. In addition to the commonly induced granulomatous inflammatory changes in the bladder, which produce irritative symptoms, this therapy may cause systemic side effects varying from mild malaise and fever to, in rare instances, life-threatening or fatal sepsis (15–17). Recovery of this strain in sputum should be recognized as such and not mistaken as *Mycobacterium tuberculosis.*

Human infection with *Mycobacterium bovis* is rare in developed countries because of milk pasteurization and the slaughter of infected cattle. While many of us may be aware that *Mycobacterium bovis* was once a major cause of tuberculosis in industrialized countries, most are unaware that this mycobacterium has never been totally eradicated (64, 80, 81) and that it has always been a cause of tuberculosis in certain immigrant groups and elderly people. The recognition of its continued role is not just an academic exercise, since its isolation has additional public health implications (64).

Because of these trends, there is an increasing need for the clinical microbiology laboratory to recognize *Mycobacterium bovis* or *Mycobacterium bovis* [BCG] isolates within *Mycobacterium tuberculosis* complex. A negative nitrate reduction and a negative niacin accumulation test are well known characteristics of these two members of the complex. Thiophen-2-carboxylic acid hydrazide susceptibility separates *Mycobacterium bovis* and *Mycobacterium bovis* [BCG] from *Mycobacterium tuberculosis*, which is resistant. Pyrazinamide monoresistance may serve as an additional marker for recognizing *Mycobacterium bovis* and *Mycobacterium bovis* [BCG] (M. Salfinger et al., 90th Annual Meeting of the American Society for Microbiology, Anaheim, 1990, Abstract no. U-55). Thus, pyrazinamide resistance generally indicates the need for a more extended test battery for the identification of isolates in the *Mycobacterium tuberculosis* complex, i.e., HPLC (77) or oxygen preference (82, 83). While *Mycobacterium bovis* grows microaerophilically in a semisolid medium, *Mycobacterium tuberculosis* and *Mycobacterium bovis* [BCG] are both aerobic.

Antimicrobial Susceptibility Testing

At present, there are three well recognized methods for determining antimicrobial susceptibility, all of which have been described elsewhere (84), namely, the proportion method, the absolute

concentration technique and the resistance ratio method. Most laboratories use a modified proportion method according to the proposed standard of the National Committee for Clinical Laboratory Standards (NCCLS) (85). Drugs are either delivered by solution or disks into a solid media, or, increasingly, susceptibility testing is performed with the faster Bactec broth method (Figure 1). Resistance is defined by growth on drug-containing media that represents ≥ 1 % of the colonies observed on the drug-free medium. The Bactec system generates results for the primary antituberculosis drugs most rapidly. After a radiometric screen against the first-line drugs – streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide – conventional agar techniques can be used to obtain additional susceptibility data for those isolates resistant to one or more of the primary drugs. Caution must be exercised to ensure that mixed cultures of *Mycobacterium tuberculosis* and nontuberculous mycobacteria are not overlooked. This can result in an apparent resistance, especially when the inoculum is derived from a broth. The evaluation of second-line drugs and experimental compounds in the Bactec system is now being studied by several laboratories and will be available in the not too distant future (M. Salfinger et al., 94th General Meeting of the American Society for Microbiology, Las Vegas, 1994, Abstract no. U-138).

In view of the increase of multi-drug resistant tuberculosis in certain areas it has been recommended by various institutions that isolates from *all* patients undergo drug susceptibility testing and that patients preferably receive a four-drug regimen until the results of susceptibility testing are known (2). In settings with a high prevalence of drug resistance (86, 87), direct susceptibility testing may even be performed from smear-positive specimens (30). This method, however, is poorly standardized and contamination may occur.

In contrast to tubercle bacilli, susceptibility testing of nontuberculous mycobacteria is a very controversial matter: it is not standardized at all and there are, so far, no reliable data reporting a correlation of the in vitro results with the in vivo situation (84; data to be published). As a general principle, in vitro susceptibility testing of slow-growing nontuberculous mycobacteria should be omitted. When requested explicitly by the physician, testing should only be done by experienced laboratories for clinically significant isolates of *Mycobacterium kansasii* (88, 89) or *Mycobacterium marinum* (88). Results must be interpreted with caution. Susceptibility testing of disseminated *Mycobacterium avium* complex in patients infected with HIV is similarly biased because there are severe doubts about the usefulness of such studies in view of therapeutic efficacy (14, 90).

For the rapidly growing mycobacteria, routine tests include a disk diffusion method and a broth microdilution technique (84; data to be published). Tests are similar to those used in bacteriology. The results are available within 72 h.

Ib. The Challenge: New Techniques for Direct Detection, Identification, Susceptibility Testing, and Epidemiological Investigations

Gas-Liquid Chromatography/Mass Spectrometry

The diagnosis of tuberculous meningitis still represents a major problem in mycobacteriology (51), since the traditional techniques of detecting AFB in cerebrospinal fluid are very unsatisfactory: microscopy is insensitive (56), and culture is a slow and low-yield procedure (55). At present, no data are available for the more sensitive radiometric cultivation technique that would allow an adequate comparison of the two methodologies.

Thus, the introduction of GLC/MS for the detection of tuberculostearic acid (R-10 methyl octade canoic acid; TBSA), a structural component of *Mycobacterium* as well as *Nocardia* species and certain other aerobe gram-positive rods, represented a major breakthrough in the direct analysis of cerebrospinal fluid (91). TBSA is detected by GLC/MS combined with selected ion-monitoring at *m/z* (mass/charge ratio) = 74, 167, and 312. The first signal is obtained for any fatty acid molecule, the second one indicates a methylated side chain (fragment ion), and the third one represents a C_{18} methyl ester (molecule ion). Identification of TBSA is based on the simultaneous presence of the three signals, exhibiting thus, an identical retention time. Larsson et al. (18) have shown as early as 1979 that selected ion-monitoring allows quite a specific identification of the compound and dramatically increases the sensitivity of detection compared with total ion monitoring or conventional GLC.

In the pioneer study of French et al. (91) TBSA could be detected in the cerebrospinal fluid from 13 patients with proven tuberculous meningitis

and from eight of nine patients with suspected tuberculous meningitis. There was one false-positive result in the control group, which consisted of 87 patients with non-tuberculous meningitis or non-infectious disorders. Remarkably, of these 22 cases, only eight yielded *Mycobacterium tuberculosis* in culture. Unfortunately, at that time, only solid media were used.

Compared with the original procedure, the extraction as well as the methylation step for the detection of TBSA has recently been modified in such a way that this technique can now easily be applied in a clinical mycobacteriology laboratory (Adler et al., Swiss National Center for Mycobacteria, Zurich, unpublished data). Standardization was achieved by injecting synthetic TBSA and by analyzing cerebrospinal fluid specimens that had been artificially spiked with various amounts of *Mycobacterium tuberculosis* cells. Under these conditions, the detection limit of the methyl ester is approximately 10 pg, which corresponds to the amount of TBSA released from approximately 10^3 AFB. Using this modified procedure in a prospective study, clinical cerebrospinal fluid specimens (n = 333) have been analyzed. TBSA was found in 22 specimens, seven of which yielded a positive culture as well (media used: LJ slant, Middlebrook 7H10/sel7H11 agar and Bactec). Eight of the remaining 15 TBSA-positive specimens were culture-negative but originated from patients showing clinical symptoms that were highly consistent with tuberculous meningitis; the other seven were false-positive. Apart from two false-negative results (TBSA negative/culture positive), all other cerebrospinal fluid specimens (n = 309) were negative by either technique. Compared with culture and clinical data, detection of TBSA by GLC/MS showed a sensitivity of 88.2 %, a specificity of 97.8 %, a positive predictive value of 68.1 % and a negative predictive value of 99.4 % (Adler et al., unpublished data).

Nevertheless, the sensitivity of the detection of TBSA by GLC/MS varies greatly with the type of clinical specimen to be analyzed. For sputum a sensitivity of between 90 % (92) and 97.1 % (93) was found, while for pleural effusion and bronchial washing fluid the sensitivity dropped to 75 % and 68 %, respectively (92), when compared with culture in liquid (Dubos) and on solid (Ogawa) media.

Although the detection of TBSA by GLC/MS is much faster (results are available within 24 h upon receipt of the specimen) and obviously more sensitive than the cultural approach on solid media, this technique is very demanding with regard to equipment and its maintenance. This, together with the fact TBSA is not specific for *Mycobacterium tuberculosis* only, may, at least to some extent, explain why this methodology is still not applied widely.

Direct Detection of Acid-Fast Bacilli in Clinical Specimens by Amplification

The application of molecular biological techniques has led to dramatic progress in the rapidity and accuracy of tuberculosis diagnostics. Although DNA probes have become an indispensable tool for today's clinical mycobacteriology laboratory, they are not sensitive enough for direct detection of AFB in patient specimens, since 10^3 to 10^4 microorganisms are required (94). Significant advances in tuberculosis diagnostics have been achieved by amplification techniques targeting either DNA or RNA. Currently, most experience is available from the direct detection of *Mycobacterium tuberculosis* in clinical specimens by the polymerase chain reaction (PCR; for refs., see 95).

Eisenach et al. have used the *Mycobacterium tuberculosis* complex specific repeated DNA elements IS*6110* as target sequence (96, 97), while Brisson-Noël et al. (98) have chosen the 65 kDa gene to detect tubercle bacilli by PCR. Although some excellent procedures for the detection of *Mycobacterium tuberculosis* in respiratory specimens by PCR have now been developed which yield both high sensitivity and specificity [83.5 %/99.0 % (99); 93.9 %/94.3 % (100); 87.2 %/97.7 % (101)], there are still authors reporting disappointingly insensitive assays as well [55 % and 74 %/98 % and 95 %, depending on the detection procedure: (102)]. Apparently most difficulties arise from microscopically negative specimens [sensitivity 57 % (103)]. Schluger et al. evaluated the clinical utility of PCR in the diagnosis of infections due to *Mycobacterium tuberculosis* at Bellevue Hospital in New York (104). Sixty-five patients were enrolled, and PCR was positive in 37 patients. When correlated with smear, culture, pathology and clinical history, the sensitivity of PCR for diagnosis of tuberculosis disease was 100 %. The specificity was, however, only 70 %, since the PCR assay was positive in a number of patients also with prior tuberculosis, treated disease, or tuberculosis infection.

Data are still scarce on clinical specimens of non-respiratory origin. De Wit et al. (105) compared

conventional methods (culture on solid media and Bactec) with PCR to diagnose tuberculous pleural effusion. The sensitivity of culture from pleural fluid was 53 %, but PCR was significantly better (sensitivity 81 %) and certainly superior to pleural biopsy culture and histology. PCR also appears to be superior to conventional bacteriology and an enzyme immunosorbent assay (EIA) for cerebrospinal fluid antibodies in the diagnosis of tuberculous meningitis (106) although this technique only detected 75 % of the highly probable cases as based on clinical features.

As evidenced by a recent interlaboratory study using a coded panel of samples containing various numbers of *Mycobacterium bovis* [BCG] cells, the reliability of PCR in detecting *Mycobacterium tuberculosis* complex remains questionable, since unexpected high variations in sensitivity among the laboratories were observed (107). It has also been shown that the implementation of an effective system for monitoring sensitivity and specificity is required in the clinical mycobacteriology laboratory before PCR can be used reliably in the diagnosis of tuberculosis (108). Similarly, many of the protocols do not fit easily into a clinical laboratory's work flow, since they can be quite demanding as far as technical equipment and operational skills are concerned.

Before long, a PCR kit that may overcome such difficulties should be commercially available (Roche Diagnostic Systems, USA). The assay's target is the 16S rRNA gene, within which two primers identify a 583-bp amplicon, being specific for more than 30 mycobacterial species. Since the 16S rRNA gene also contains hypervariable regions, further identification is possible using species-specific probes following a positive genus screen.

A recently developed isothermal transcription-mediated amplification system detects *Mycobacterium tuberculosis* complex directly from respiratory specimens and provides a billion-fold amplification of the rRNA targets. In this test system (Amplified *Mycobacterium tuberculosis* Direct Test [MTD] Gen-Probe, USA), amplicons are created via DNA intermediates and subsequently detected by the same hybridization protection assay employed in the conventional Gen-Probe tests (AccuProbe) for culture confirmation. Jonas et al. (19) obtained an overall sensitivity of 80 % and a specificity of 97 % with the Amplified Gen-Probe assay on induced sputum specimens (n = 758). After review of the patients' clinical data, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 82 %, 99 %, 97 % and 96 %, respectively, for the MTD, and 88 %, 100 %, 100 % and 97 % for culture. The low sensitivity achieved for culture is not surprising because only two LJ slants but no liquid (radiometric) media were used. Similar values are reported from Abe et al. (109) and Miller et al. (20). Pfyffer et al. (34) have evaluated a total of 938 respiratory specimens by the MTD and compared the results with fluorescence microscopy and cultivation (LJ slant plus Middlebrook 7H10/sel7H11 plus Bactec). One series of the specimens was decontaminated with NALC/NaOH, the other one with SDS/NaOH. Overall sensitivity and specificity of the MTD were 92.9 % and 96.2 %, respectively, after NALC decontamination; and 97.2 % and 96.1 %, respectively, after SDS treatment. Analysis of the discordant results revealed that quite a few MTD positive/culture negative specimens could be considered true positives after review of the patients' clinical data. After resolution of the discrepancies, the following values were obtained: 93.9 % sensitivity/97.6 % specificity for the NALC series, and 97.4 % sensitivity/96.9 % specificity for the SDS series. These data demonstrate that the MTD works slightly though not significantly better ($p > 0.05$) for SDS-decontaminated specimens, provided that the sediments are properly washed prior to the MTD (34).

The high reliability of this new test makes it an ideal tool for diagnosing tuberculosis with low bacterial load. Moreover, with a negative predictive value of almost 100 %, the test can quickly help exclude tuberculosis from the differential diagnosis. When the specimen is microscopically positive but MTD negative, the test may point to the presence of nontuberculous mycobacteria.

Alternative amplification procedures aiming also at the direct detection of *Mycobacterium tuberculosis* in clinical specimens are currently in the process of development or in early stages of clinical evaluation and should be available in kit form before long. Among them are strand-displacement amplification (110), nucleic-acid-sequence-based amplification (111), ligase chain reaction (112), and Qβ replicase amplification (113).

Present Value of Molecular Test Systems Used for the Direct Detection of Mycobacterium tuberculosis in the Routine Diagnostic Laboratory

Certainly, the molecular biological technology has opened new dimensions in tuberculosis diag-

nostics as far as sensitivity, specificity, and speed are concerned. However, difficulties in the interpretation of results produced by any of these highly sensitive methods become obvious when results diverge from those obtained by conventional culture and identification procedures. Further evaluations must, therefore, be performed to answer questions such as (i) false positives: reliability of the presently used 'gold standard' (e.g. culture); (ii) false negatives: possible presence of amplification inhibitors in the specimen; (iii) types of cells detected by molecular methods: viable cells only, or viable cells as well as dead ones; (iv) clinical interpretation of a positive result: acute infection, reactivation of disease, or simply persisting (inactive) population of tubercle bacilli; and (v) initiation/onset of antituberculous/preventive chemotherapy, etc.

At present, results obtained by any of the new molecular methods should be interpreted in conjunction with the patient's clinical data, i.e., medical history, physical examination, chest radiograph, tuberculin skin test, and contemporary techniques for microscopy, culture, and identification. Thus, it must be stressed that both clinician and microbiologist have to meet the results generated by these new methods with the necessary skepticism and that an optimum collaboration is critical to a positive outcome for the patient.

Species Identification of Acid-Fast Bacilli

In mycobacteria, well-preserved, genus-specific regions within the 16S rRNA co-occur, with hypervariable regions exhibiting species-specific characteristics (114). Kirschner et al., (115) have identified quite successfully 473 clinical isolates of *Mycobacterium* spp. by direct sequence determination of 16S rRNA gene fragments amplified by PCR and have compared the results with those of traditional biochemical testing. PCR-mediated direct sequence determination allowed, in addition, prompt recognition of previously undescribed species, i.e. isolates that could not be identified by standard biochemical tests (e.g., *Mycobacterium confluentis*, *Mycobacterium genavense*, *Mycobacterium intermedium*, *Mycobacterium interjectum*). This technique cannot, however, differentiate between *Mycobacterium kansasii* and *Mycobacterium gastri* or between *Mycobacterium marinum* and *Mycobacterium ulcerans*. Because of workload and practicability problems, this methodology may have to be limited to reference laboratories rather than to routine clinical laboratories.

Plykaytis et al. (116) and Telenti et al. (117) described a rapid method for identification of mycobacteria to the species level through PCR amplification of 65 kDa heat shock protein gene sequences combined with restriction endonuclease analysis. Lungu et al. (118) amplified DNA from groEL gene and through restriction-fragment-length polymorphism analysis were successful in the differentiation of *Nocardia* from rapidly growing mycobacteria. PCR combined with restriction-fragment-length polymorphism is less expensive and less labor-intensive than sequencing and has the potential to be implemented in a busy clinical microbiology laboratory.

Future Possibilities in Susceptibility Testing

Independent of the emergence of multi-drug resistant tuberculosis in certain areas, rapid assessment of the results of drug susceptibility testing of *Mycobacterium tuberculosis* isolates has become of prime importance. Several exciting approaches have been elaborated very recently to overcome the problem of putting patients, contacts, and health care workers at risk. Some of them improve simply the understanding of the mechanisms of drug resistance, while others may lead to the development of rapid tests which should, in the near future, allow their use and application in a routine diagnostic laboratory (119–127).

DNA Typing

For epidemiological studies (e.g., nosocomial transmission, reactivation of tuberculosis vs. reinfection, cross-contamination in the laboratory, etc.), biochemical strain properties, unusual antibiotic susceptibility patterns, or even phage typing have considerable limitations. A few years ago, repetitive DNA elements were identified in *Mycobacterium tuberculosis* and their potential for use as genetic markers recognized (128, 129). In contrast to the limited polymorphism of the restriction fragments of the insertion sequence (IS) *1081* (130), the number of IS*6110* copies and the sites of insertion in the chromosome are highly variable. Thus, the IS*6110* element is extremely powerful and, therefore, is being used most widely as a DNA probe in the epidemiological

distinction of *Mycobacterium tuberculosis* strains. Cave et al. (131) have reported good stability of the IS*6110* fingerprints during periods of up to 4.5 years and, in addition, reported that acquisition of drug resistance was not associated with pattern changes. Recently, recommendations for a standardized DNA typing methodology have appeared (132).

Major applications of DNA fingerprinting include epidemics in homeless shelters (133) and nosocomial outbreaks in HIV facilities (1) or in renal transplant units (134). In the latter case, rapid DNA typing of IS*6110* was performed by PCR using a modified single-site PCR method specific for this insertion sequence (135).

In a population-based study using conventional and molecular methods, the epidemiology of tuberculosis in San Francisco was analyzed (136). Despite an efficient tuberculosis control program, nearly one-third of new cases of tuberculosis are the result of a recent infection. Few of these instances of transmission have been identified by conventional contact tracing. The San Francisco experience suggests that current tuberculosis control strategies have important limitations in contemporary urban environments.

The isolation of even a single positive culture for *Mycobacterium tuberculosis* complex should be the basis of a presumptive diagnosis of tuberculosis. A false-positive culture may have profound consequences on the clinical management of the patient. Episodes of laboratory cross-contamination may occur more frequently than may be recognized in the current resurgence of tuberculosis. In a recent study of cross-contamination, the potential of molecular strain typing was demonstrated (137). In this investigation, the impact of false-positive cultures was significant, even though it was mitigated by early recognition that these were probably the result of laboratory cross-contamination. Patients with false-positive cultures were nonetheless subjected to additional physician consultations, radiographic studies and acquisition of specimens for AFB workup. An additional disturbing consequence of these spurious results was the unnecessary administration of antituberculosis therapy to patients and contacts, resulting in adverse drug reactions in two patients (137). There is no justification for fingerprinting of *Mycobacterium tuberculosis* strains on a routine basis; however, the ultimate role of such procedures in clinical laboratory practice for quality control purposes may be significant to be determined.

II. The Levels of Service Concept

Laboratory directors take pride in the scope and precision of the tests performed in their institutions. However, it is the physician's responsibility to provide adequate care for the patient. On the basis of the physician's knowledge, laboratory tests are chosen primarily to screen for unsuspected disease and to establish or exclude tuberculosis or other mycobacterial disease. With the closing of the sanatoria and the treatment of patients in general hospitals or on an outpatient basis, the supporting mycobacteriology service has spread diffusely through a growing number of laboratories offering different extents and qualities of laboratory service.

IIa. Current Situation

Suggestions for several levels or extents of services that might be provided by mycobacteriology laboratories have been proposed by the Centers for Disease Control and Prevention, the American Thoracic Society, and the College of American Pathologists. It is recommended in the levels-of-service concept, first introduced in 1967 by the Public Health Service (138) and supported by the American Thoracic Society in official policy statements (139-141), that services be based on work load, expertise and cost-effectiveness.

The current three-level concept of laboratory services for mycobacterial diseases (Level I: smear; Level II: in addition to I, culture, identification of *Mycobacterium tuberculosis* complex; Level III: as in II, plus susceptibility testing and identification of all mycobacteria) has been reiterated by the International Union Against Tuberculosis and Lung Disease and the World Health Organization in 1990 (142). On the basis of a workshop concerned with tuberculosis elimination in European and other industrialized countries, it was recognized that the elimination of tuberculosis will demand diligent work for decades to come, particularly in light of the HIV pandemic. It is essential that centers of expertise be maintained or established where they do not currently (or no longer) exist. These centers should play a leading role in developing programs to improve surveillance and should also act as a resource offering service guidance, training and support for all health care workers dealing with tuberculosis. In their prospective organization of

laboratory services for tuberculosis in low incidence countries, three levels are again mentioned: Level I, smear only; Level II, smear, culturing and identification for tuberculosis only; and Level III, identification of all mycobacterial species along with susceptibility testing. While Level I laboratories are local, Level II laboratories render service for 1-4 million inhabitants, and Level III laboratories for 5-10 million inhabitants or nationally. In addition, Level III laboratories carry out studies on drug resistance and the development and evaluation of new technology.

The current concept of laboratory services for mycobacterial diseases faces new challenges in this era of increased numbers of immunodeficient patients and of those with multidrug-resistant tuberculosis. The results not only must be accurate; they must be generated in a timely fashion as well. The hierarchic principle, i.e., waiting for well-grown subcultures to mail out, is responsible for delays. From this, it appears that the Level II laboratories are the weakest link in the chain. Some of these laboratories are using solid media only; they may identify tubercle bacilli without probe technology, and they may forward isolates to laboratories that are performing drug susceptibility testing only with solid media. Contributing to a long turnaround time, many of the laboratories involved are staffed only five days a week.

IIb. Fast-Track Program – A Model Concept

Motivated by the equipment and training costs necessary to upgrade many mycobacteriology laboratories to Level III, the New York State Department of Health has introduced a 'Fast Track' program for tuberculosis testing. The goal of this program is to provide statewide susceptibility testing results for highly contagious smear-positive patients to the clinician as soon as possible, at the latest before patients are discharged from the hospital, so that appropriate therapy may be initiated immediately. This program relies on the use of proven rapid technologies (radiometric detection, species-specific nucleic-acid-hybridization identification, and radiometric susceptibility testing); rapid specimen transport by overnight courier to a central laboratory; same-day reporting by fax; and seven-days-per-week service. Preliminary results show that susceptibility test results are available to the clinician in less than three weeks after sputum collection for about 80 % of the smear-positive cases. While such a program may not be feasible for many laboratories, the results demonstrate that mycobacteriology services can be provided in an expeditious manner, resulting in timely information that has a meaningful impact on clinical care and public health.

IIc. Dynamic Acid-Fast Network

If patient care and public health are always considered paramount regardless of admission time, hospital type, geographical region, etc., the current concept of services has several shortcomings: the laboratory may be slow in the overall turnaround time and therefore, not cost-efficient. In addition, an unknown number of false-negative specimens may render the quality of laboratory service below expectations.

One way to deal with the current situation might be to sort and allocate specimens according to a system of priorities. The model 'Fast Track' program for tuberculosis testing as part of a new acid-fast network (Figure 2) focuses on the highly infectious patient population. The required screening step is an economic way to utilize limited resources. The 'Fast Track' laboratory has three goals: first, the fastest and most reliable technologies should be implemented to facilitate the shortest turnaround times possible for susceptibility testing; second, centralization of such services will assist in controlling costs; and third, patients with nontuberculous mycobacterial diseases (mycobacteriosis) will stay in respiratory isolation rooms for shorter periods of time and may discontinue anti-tuberculosis drugs earlier.

The impact of the initial screening by smear may be improved by performing the smear in-house (the 'Point of Care' laboratory, Figure 2) or at least at courier distance, allowing less than 24-hour turnaround time from taking of that particular specimen. A widespread use of cytocentrifuge technology for concentrating specimens may increase the yield of smear-positive patients.

The third or 'Specialty' laboratory (Figure 2), is the most versatile and – based on the tests performed – the most expensive one in the dynamic acid-fast network. With the current methodologies available, this laboratory could cover one or more of the following topics: testing of second-line and experimental drugs for strains of *Mycobacterium tuberculosis* complex, susceptibility testing for clinically significant isolates of rapid growers, *Mycobacterium kansasii* or other slow-

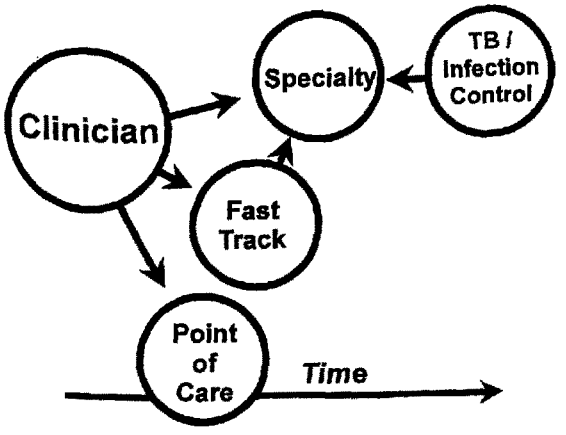

Figure 2: Dynamic acid-fast network. A novel approach stresses the physician's role in diagnosing tuberculosis. Three laboratory types are partners in the dynamic acid-fast network: Point-of-Care, Fast Track, and Specialty (see text). Arrows mark sites that request laboratory testing. The clinician interacts with all three types of laboratories.

growing nontuberculous mycobacteria; identification of mycobacteria utilizing high-performance liquid chromatography, PCR coupled with restriction-fragment-length-polymorphism analysis, or sequencing DNA found in smear-positive specimens; DNA typing of tubercle bacilli or pulsed-field gel electrophoresis of *Mycobacterium avium* complex strains and other clinically significant species; detection of tuberculostearic acid in cerebrospinal fluid of patients with suspicion of tuberculous meningitis; determination of serum concentrations for anti-tuberculosis drugs; and evaluation of new diagnostic kits. This highly sophisticated type of laboratory may serve a metropolitan area, an entire region, or even several countries, e.g., in Europe.

It is recommended that the sites involved in the dynamic acid-fast network – Point of Care, Fast Track, and Specialty – offer seven-day-per-week service to minimize delays that might be of organizational nature. It should also be noted that the use of the dynamic acid-fast network should be requested by an infectious disease physician on duty or another expert in the field. The implementation of this additional dynamic acid-fast network will have a meaningful impact on both clinical care and on public health.

III. Reporting

Early reporting of tuberculosis is essential for prompt evaluation of contacts to the source case. By law, any case of tuberculosis must be reported to the local and, in certain countries, to the national public health authorities as well. In the USA, reporting of the initial results of susceptibility testing is mandated to facilitate surveillance of drug-resistant tuberculosis (2). The same is planned in European countries such as Switzerland.

The laboratory itself must make an extra effort for rapid reporting, particularly when taking into account that laboratory procedures in mycobacteriology are still much more time consuming than those for other bacteria, despite the latest developments in the rapid diagnosis of mycobacterial disease. Whether tubercle bacilli or nontuberculous mycobacteria will eventually be isolated, interim reports should be sent out to the physician whenever new data become available. This holds for smear results, direct detection methods (GLC/MS, amplification procedures), culture, tentative species or group identification by preliminary tests (such as the NAP test or DNA probes), and susceptibility testing. Furthermore, it is good laboratory practice to call the physician when a clinical specimen is found positive for the first time (e.g., smear, *Mycobacterium tuberculosis* complex, or resistant tubercle bacilli). This does, of course, not free the laboratory from a written interim report.

If the mycobacterial species has definitely been identified and the data from the susceptibility testing are available, a final report containing all the data previously reported is issued and sent to the physician. Fax copies may expedite reporting. However, confidentiality must be guaranteed.

IV. Teamwork

In recent years there has been a growing body of new exciting methods in mycobacteriology. At the same time, however, there is also growing insight that no single method by itself is the best. Thus, several techniques should always be used, since they are complementary to each other. The laboratory director needs to decide which tests will be performed in-house and which specimens will be mailed to a specialized laboratory within the dynamic acid-fast network, based on the community to be served and the availability of resources. This decision should be made in consultation with infectious disease, pulmonary, or other physicians affected.

Although many factors contribute to and compound the escalating health care cost problem, the increased utilization of health services, especially diagnostic services, is considered one of the most important elements. From the perspective of the microbiology laboratory, there is a direct relationship between workload and the number of technologists needed to perform it as a result of the general lack of automation in the clinical microbiology laboratory (143). With limited resources available, only a few laboratories may be able to use molecular techniques as screening methods to rule out tuberculosis. Therefore, hospital staff (in-house) and laboratorians together with their clients (extra-mural) should develop policies and guidelines for utilization of these newer techniques. Direct testing of sputum may be suitable or even necessary in the following patients: smear-positive patients; smear-negative patients under treatment; patients in respiratory isolation; HIV-positive individuals; patients for whom infectious disease or pulmonary clinicians on duty make requests (i.e., markers as foreign-born, history of tuberculosis, abnormal chest radiograph, etc.). It should be emphasized that this list may differ from laboratory to laboratory because of the differences of the community served.

Returning to the five clinical scenarios initially mentioned – 1) highly infectious patients with acid-fast smear-positive pulmonary tuberculosis; 2) tuberculous meningitis; 3) cervical unilateral lymphadenitis in infants; 4) mycobacteremia in HIV-positive patients, and 5) dissemination after intravesical instillation of *Mycobacterium bovis* [BCG] – a question arises: can your laboratory provide accurate results in a timely manner in all of these scenarios? With this proposed novel approach it should be possible.

In conclusion, several surveys have demonstrated that the implementation of new techniques in the clinical microbiology laboratory is slow and hesitant. We must work together on a dynamic acid-fast network to improve the service rendered for our patients. Excitement over well-proven molecular tools should inspire the laboratory to make the necessary changes now, so that future challenges can be met.

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References

1. **Daley CL, Small PM, Schecter GF, Schoolnik GK, McAdam RA, Jacobs WR, Hopewell PC:** An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus – an analysis using restriction-fragment-length polymorphisms. New England Journal of Medicine 1992, 326:231–235.
2. **Centers for Disease Control and Prevention:** Initial therapy for tuberculosis in the era of multidrug resistance – recommendations of the Advisory Council for the Elimination of Tuberculosis. Morbidity and Mortality Weekly Report 1993, 42 (RR-7): 1–8.
3. **Middlebrook G, Cohn ML:** Bacteriology of tuberculosis: laboratory methods. American Journal of Public Health 1958, 48: 844–853.
4. **Ellner PD, Elbogen S:** Modern methods in tuberculosis bacteriology for the general hospital. American Journal of Clinical Pathology 1967, 48: 435–440.
5. **Huebner RE, Good RC, Tokars JI:** Current practices in mycobacteriology: results of a survey of state public health laboratories. Journal of Clinical Microbiology 1993, 31: 771–775.
6. **Woods GL, Witebsky FG:** Current status of mycobacterial testing in clinical laboratories – results of a questionnaire completed by participants in the College of American Pathologists Mycobacteriology E survey. Archive of Pathology and Laboratory Medicine 1993, 117: 876–884.
7. **Humphries M:** The management of tuberculous meningitis. Thorax 1992, 47: 577–581.
8. **Parsons M:** Tuberculous meningitis, tuberculomas and spinal tuberculosis – a handbook for clinicians. Oxford University Press, Oxford, 1988.
9. **Schaad UB, Votteler TD, McCracken GH, Nelson JD:** Management of atypical mycobacterial lymphadenitis in childhood. A review based on 380 cases. Journal of Pediatrics 1979, 95: 356–360.
10. **Kew Lai K, Stottmeier KD, Sherman IH, McCabe WR:** Mycobacterial cervical lymphadenopathy-relation of etiologic agents to age. Journal of the American Medical Association 1984, 251: 1286–1288.
11. **Fabbri J, Welge-Lüssen A, Frei R, Zimmerli W:** Zervikale Lymphadenitis mit *Mycobacterium malmoense* bei einem Kind: Fallbeschreibung und Differentialdiagnose der zervikalen Lymphadenitis und Lymphadenopathien. Schweizerische Medizinische Wochenschrift 1993, 123: 1756–1761.
12. **Horsburgh CR, Selik RM:** The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). American Review of Respiratory Disease 1989, 139: 4–7.
13. **Nightingale SD, Byrd LT, Southern PM, Jockusch JD, Cal SX, Wynne BA:** Incidence of *Mycobacterium avium-intracellulare* complex bacteremia in human immunodeficiency virus-positive patients. Journal of Infectious Diseases 1992, 165: 1082–1085.
14. **Masur H:** Public Health Service Task Force on Prophylaxis and Therapy for *Mycobacterium avium* complex. New England Journal of Medicine 1993, 329: 898–904.
15. **Lamm DL, van der Meijden APM, Morales A, Brosman SA, Catalona WJ, Herr HW, Soloway MS, Steg A, Debruyne FMJ:** Incidence and treatment of complications of bacillus Calmette-Guérin intravesical therapy in superficial bladder cancer. Journal of Urology 1992, 147: 596–600.

16. **McParland C, Cotton DJ, Gowda KS, Hoeppner VH, Martin WT, Weckworth PF:** Miliary *Mycobacterium bovis* induced by intravesical bacille Calmette-Guérin immunotherapy. American Review of Respiratory Disease 1992, 146: 1330–1333.
17. **Kristjansson M, Green P, Manning HL, Slutsky AM, Brecher SM, Fordham von Reyn C, Arbeit RD, Maslow JN:** Molecular confirmation of bacillus Calmette-Guérin as the cause of pulmonary infection following urinary tract instillation. Clinical Infectious Diseases 1993, 17: 228–230.
18. **Larsson L, Mård P, Odham G, Westerdahl G:** Detection of tuberculostearic acid in biological specimens by means of glass capillary gas chromatography – electron and chemical ionization mass spectrometry, utilizing selected ion monitoring. Journal of Chromatography 1980, 182: 402–408.
19. **Jonas V, Alden MJ, Curry JI, Kamisango K, Knott CA, Lankford R, Wolfe JM, Moore DF:** Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. Journal of Clinical Microbiology 1993, 31: 2410–2416.
20. **Miller N, Hernandez SG, Cleary TJ:** Evaluation of Gen-Probe amplified Mycobacterium direct test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. Journal of Clinical Microbiology 1994, 32: 393–397.
21. **Debrunner M, Salfinger M, Brändli O, von Graevenitz A:** Epidemiology and clinical significance of nontuberculous mycobacteria in patients negative for human immunodeficiency virus in Switzerland. Clinical Infectious Diseases 1992, 15: 330–345.
22. **Cox JN, Brenner ER, Bryan CS:** Changing patterns of mycobacterial disease at a teaching community hospital. Infection Control and Hospital Epidemiology 1994, 15: 513–515.
22. **Allen JL:** A modified Ziehl-Neelsen stain for mycobacteria. Medical Laboratory Sciences 1992, 49: 99–102.
24. **Urbanczik R:** Present position of microscopy and of culture in diagnostic mycobacteriology. Zentralblatt für Bakteriologie und Hygiene A 1985, 260: 81–87.
25. **Lipsky BA, Gates J, Tenover FC, Plorde JJ:** Factors affecting the clinical value of microscopy for acid-fast bacilli. Reviews of Infectious Diseases 1984, 6: 214–222.
26. **Gordin F, Slutkin G:** The validity of acid-fast smears in the diagnosis of pulmonary tuberculosis. Archives of Pathology and Laboratory Medicine 1990, 114: 1025–1027.
27. **Saceanu CA, Pfeiffer NC, McLean T:** Evaluation of sputum smears concentrated by cytocentrifugation for detection of acid-fast bacilli. Journal of Clinical Microbiology 1993, 31: 2371–2374.
28. **Bennedsen J, Larsen SO:** Examination of tubercle bacilli by fluorescence microscopy. Scandinavian Journal of Respiratory Diseases 1966, 47: 114–120.
29. **Strong BE, Kubica GP:** Isolation and identification of *Mycobacterium tuberculosis* – a guide for the level II laboratory. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, 1981.
30. **Kent PT, Kubica GP:** Public health mycobacteriology – a guide for the level III laboratory. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, 1985.
31. **Gruft H:** Evaluation of mycobacteriology laboratories: the acid-fast smear. Health Laboratory Science 1978, 15: 215–220.
32. **Tacquet A, Tison F:** Nouvelle technique d'isolement des mycobactéries par le lauryl-sulfate de sodium. Annales de l'Institut Pasteur 1961, 100: 676–680.
33. **Salfinger M, Kafader FM:** Comparison of two pretreatment methods for the detection of mycobacteria of BACTEC and Loewenstein-Jensen slants. Journal of Microbiological Methods 1987, 6: 315–321.
34. **Pfyffer GE, Kissling P, Wirth R, Weber R:** Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. Journal of Clinical Microbiology 1994, 32: 918–923.
35. **Yajko DM, Nassos PS, Sanders CA, Gonzalez PC, Reingold AL, Horsburgh CR, Hopewell PC, Chin DP, Hadley WK:** Comparison of four decontamination methods for recovery of *M. avium* complex from stools. Journal of Clinical Microbiology 1993, 31: 302–306.
36. **Roberts GD, Koneman EW, Kim YK:** *Mycobacterium.* In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ (ed.): Manual of clinical microbiology. American Society for Microbiology, Washington DC, 1991, p. 304–339.
37. **Salfinger M, Demchuk BS, Kafader FM:** Comparison between the MB Chek system, radiometric, and conventional methods for recovery of mycobacteria. Journal of Microbiological Methods 1990, 12: 97–100.
38. **Isenberg HD, D'Amato RF, Heifets L, Murray PR, Scardamaglia M, Jacobs MC, Alperstein P, Niles A:** Collaborative feasibility study of a biphasic system (Roche Septi-Chek AFB) for rapid detection and isolation of mycobacteria. Journal of Clinical Microbiology 1991, 29: 1719–1722.
39. **Abe C, Hosojima S, Fukasawa Y, Kazumi Y, Takahashi M, Hirano K, Mori T:** Comparison of MB-Check, BACTEC, and egg-based media for recovery of mycobacteria. Journal of Clinical Microbiology 1992, 30: 878–881.
40. **Roberts GD, Goodman NL, Heifets L, Larsh HW, Lindner TH, McClatchy, JK, McGinnis MR, Siddiqi SH, Wright P:** Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear-positive specimens. Journal of Clinical Microbiology 1983, 18: 689–696.
41. **Morgan MA, Horstmeier CD, DeYoung DR, Roberts GD:** Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. Journal of Clinical Microbiology 1983, 18: 384–388.
42. **Kiehn TE, Cammarata R:** Comparative recoveries of M. *avium-M. intracellulare* from isolator lysis-centrifugation and BACTEC 13A blood culture systems. Journal of Clinical Microbiology 1988, 26: 760–761.
43. **Witebsky FG, Keiser JF, Condville PS, Bryan R, Park CH, Walker R, Siddiqi SH:** Comparison of BACTEC 13A medium and Du Pont Isolator for detection of mycobacteremia. Journal of Clinical Microbiology 1988, 26: 1501–1505.
44. **Salfinger M, Stool EW, Piot D, Heifets L:** Comparison of three methods for recovery of *Mycobacterium avium* complex from blood specimens. Journal of Clinical Microbiology 1988, 26: 1225–1226.
45. **von Reyn FC, Hennigan S, Niemczyk S, Jacobs NJ:** Effect of delays in processing on the survival of *Mycobacterium avium-M. intracellulare* in the Isolator blood culture system. Journal of Clinical Microbiology 1991, 29: 1211–1214.

46. **Wasilauskas B, Morrell B, Jr:** Inhibitory effect of the Isolator blood culture system on growth of *Mycobacterium avium-M. intracellulare* in BACTEC 12B bottles. Journal of Clinical Microbiology 1994, 32: 654–657.
47. **Askgaard D, Fuursted K, Gottschau A, Bennedsen J:** Detection of mycobacteria from blood and bone marrow: a decade of experience. Acta Pathologica, Microbiologica, et Immunologica Scandinavica 1992, 100: 609–614.
48. **Havlir D, Kemper CA, Derenski SC:** Reproducibility of lysis-centrifugation cultures for quantification of *Mycobacterium avium* complex bacteremia. Journal of Clinical Microbiology 1993, 31: 1794–1798.
49. **Yagupsky P, Menegus MA:** Cumulative positivity rates of multiple blood cultures for *Mycobacterium avium-intracellulare* and *Cryptococcus neoformans* in patients with the acquired immunodeficiency syndrome. Archives of Pathology and Laboratory Medicine 1990, 114: 923–925.
50. **Stone BL, Cohn DL, Kane MS, Hildred MV, Wilson ML, Reves RR:** Utility of paired blood cultures and smears in diagnosis of disseminated *Mycobacterium avium* complex infections in AIDS patients. Journal of Clinical Microbiology 1994, 32: 841–842.
51. **Kent SJ, Crowe SM, Yung A, Lucas CR, Mijch AM:** Tuberculous meningitis: a 30-year review. Clinical Infectious Diseases 1993, 17: 987–994.
52. **Greenlee JE:** Approach to diagnosis of meningitis – cerebrospinal fluid evaluation. Infectious Disease Clinics of North America 1990, 4: 583–598.
53. **Bromberg K:** Policy for fungal and mycobacterial culture requests on CSF. Lancet 1980, i: 1023.
54. **Crowson TW, Rich EC, Woolfrey BF, Conelly DP:** Overutilization of cultures of CSF for mycobacteria. Journal of the American Medical Association 1984, 251: 70–72.
55. **Albright RE, Graham CB, Christinson RH, Schell WA, Bledsoe MC, Emlet JL, Mears TP, Reller LB, Schneider KA:** Issues in cerebrospinal fluid management – acid-fast bacillus smear and culture. American Journal of Clinical Pathology 1991, 95: 418–423.
56. **Kennedy DH, Fallon RJ:** Tuberculous meningitis. Journal of the American Medical Association 1979, 241: 264–268.
57. **Abadco DL, Steiner P:** Gastric lavage is better than bronchoalveolar lavage for isolation of *Mycobacterium tuberculosis* in childhood pulmonary tuberculosis. Pediatric Infectious Disease Journal 1992, 11: 735–738.
58. **Yagupsky PV, Kaminski DA, Palmer KM, Nolte FS:** Cord formation in BACTEC 7H12 medium for rapid, presumptive identification of *Mycobacterium tuberculosis* complex. Journal of Clinical Microbiology 1990, 28: 1451–1453.
59. **Morris AJ, Reller LB:** Reliability of cord formation in BACTEC media for presumptive identification of mycobacteria. Journal of Clinical Microbiology 1993, 31: 2533–2534.
60. **Evans KD, Nakasone AS, Sutherland PA, de la Maza LM, Peterson EM:** Identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-M. intracellulare* directly from primary BACTEC cultures by using acridinium-ester-labeled DNA probes. Journal of Clinical Microbiology 1992, 30: 2427–2431.
61. **Lebrun L, Espinasse F, Poveda JD, Vincent-Lévy-Frébault V:** Evaluation of nonradioactive DNA probes for identification of mycobacteria. Journal of Clinical Microbiology 1992, 30: 2476–2478.
62. **Dankner WM, Waecker NJ, Essey MA, Moser K, Thompson M, Davis CE:** *Mycobacterium bovis* infections in San Diego: a clinicoepidemiologic study of 73 patients and a historical review of a forgotten pathogen. Medicine 1993, 72: 11–37.
63. **Ephraim DA, Spitzer ED:** Use of acridinium-ester-labeled DNA probes for identification of mycobacteria in BACTEC 13A blood cultures. Diagnostic Microbiology and Infectious Disease 1994, 18: 137–139.
64. **Martin C, Vincent Lévy-Frébault V, Cattier B, Legras A, Goudeau A:** False positive result of *Mycobacterium tuberculosis* complex DNA probe hybridization with a *Mycobacterium terrae* isolate. European Journal of Clinical Microbiology and Infectious Diseases 1993, 12: 309–310.
65. **Butler WR, O'Connor SP, Yakrus MA, Gross WM:** Cross-reactivity of genetic probe for detection of *Mycobacterium tuberculosis* with newly described species *Mycobacterium celatum*. Journal of Clinical Microbiology 1994, 32: 536–538.
66. **Stockman L, Springer B, Böttger EC, Roberts GD:** *Mycobacterium tuberculosis* nucleic acid probes for rapid diagnosis. Lancet 1993, 341: 1486.
67. **Brander E, Jantzen E, Huttunen R, Julkunen A, Katila ML:** Characterization of a distinct group of slowly growing mycobacteria by biochemical tests and lipid analyses. Journal of Clinical Microbiology 1992, 30: 1972–1975.
68. **Kirschner P, Vogel U, Hein R, Böttger EC:** Bias of culture techniques for diagnosing mixed *Mycobacterium genavense* and *Mycobacterium avium* infection in AIDS. Journal of Clinical Microbiology 1994, 32: 828–831.
69. **Kiehn TE, Cammarata R:** Laboratory diagnosis of mycobacterial infections in patients with acquired immunodeficiency syndrome. Journal of Clinical Microbiology 1986, 24: 708–711.
70. **Laszlo A, Siddiqi SH:** Evaluation of a rapid radiometric differentiation test for the *Mycobacterium tuberculosis* complex by selective inhibition with p-nitro-α-acetylamino-β-hydroxypropiophenone. Journal of Clinical Microbiology 1984, 19: 694–698.
71. **Gross WM, Hawkins JE:** Radiometric selective inhibition tests for differentiation of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and other mycobacteria. Journal of Clinical Microbiology 1985, 21: 565–568.
72. **Butler WR, Ahearn DG, Kilburn JO:** High-performance chromatography of mycolic acids as a tool in the identification of *Corynebacterium*, *Nocardia*, *Rhodococcus*, and *Mycobacterium* species. Journal of Clinical Microbiology 1986, 23: 182–185.
73. **Guthertz LS, Lim SD, Jang Y, Duftey PS:** Curvilinear-gradient high-performance liquid chromatography for identification of mycobacteria. Journal of Clinical Microbiology 1993, 31: 1876–1881.
74. **Cage GD:** Direct identification of *Mycobacterium* species in BACTEC 7H12B medium by high-performance liquid chromatography. Journal of Clinical Microbiology 1994, 32: 521–524.
75. **Glickman SE, Kilburn JO, Butler WR, Ramos LS:** Rapid identification of mycolic acid patterns of mycobacteria by high-performance liquid chromatography using pattern recognition software and a *Mycobacterium* library. Journal of Clinical Microbiology 1994, 32: 740–745.

76. **Thibert L, Lapierre S:** Routine application of high-performance liquid chromatography for identification of mycobacteria. Journal of Clinical Microbiology 1993, 31: 1759–1763.
77. **Floyd MM, Silcox VA, Jones WD, Butler WR, Kilburn JO:** Separation of *Mycobacterium bovis* [BCG] from *Mycobacterium tuberculosis* and *Mycobacterium bovis* by using high-performance liquid chromatography of mycolic acids. Journal of Clinical Microbiology 1992, 30: 1327–1330.
78. **Tisdall PA, DeYoung DR, Roberts GD, Anhalt JP:** Identification of clinical isolates of mycobacteria with gas-liquid chromatography: a 10-month follow-up study. Journal of Clinical Microbiology 1982, 16: 400–402.
79. **Smid I, Salfinger M:** Mycobacterial identification by computer-aided gas-liquid chromatography. Diagnostic Microbiology and Infectious Disease 1994, 19: 81–88.
80. **Fanning A, Edwards S:** *Mycobacterium bovis* infection in human beings in contact with elk (*Cervus elaphus*) in Alberta, Canada. Lancet 1991, 338: 1253–1255.
81. **De Kantor IN, Ritacco V:** Bovine tuberculosis in Latin America and the Caribbean: current status, control and eradication programs. Veterinary Microbiology 1994, 40: 5–14.
82. **Lebek G:** Über den Nachweis des unterschiedlichen Sauerstoffoptimums des humanen und bovinen *Mycobacterium tuberculosis*. Zentralblatt für Bakteriologie (I. Abteilung Originale) 1958, 173: 581–587.
83. **Marks J:** Classification of the mycobacteria in relation to clinical significance. Tubercle 1972, 53: 259–264.
84. **Inderlied CB:** Antimycobacterial agents: in vitro susceptibility testing, spectrums of activity, mechanisms of action and resistance, and assays for activity in biological fluids. In: Lorian V (ed): Antibiotics in laboratory medicine. Williams and Wilkins, Baltimore, 1991.
85. **National Committee for Clinical Laboratory Standards:** Antimycobacterial susceptibility testing. Proposed standard M24-P. Villanova, PA, 1990.
86. **Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW:** The emergence of drug-resistant tuberculosis in New York City. New England Journal of Medicine 1993, 328: 521–526.
87. **Bloch AB, Cauthen GM, Onorato IM, Dansbury KG, Kelly GD, Driver CR, Snider DE:** Nationwide survey of drug-resistant tuberculosis in the United State. Journal of the American Medical Association 1994, 271: 665–671.
88. **Wallace RJ, O'Brien R, Glassroth J, Raleigh J, Dutt A:** Diagnosis and treatment of disease caused by nontuberculous mycobacteria. American Review of Respiratory Disease 1990, 142: 940–953.
89. **Wallace RJ, Dunbar D, Brown BA, Onyi G, Dunlap R, Ahn CH, Murphy DT:** Rifampin-resistant *Mycobacterium kansasii*. Clinical Infectious Diseases 1994, 18: 736–743.
90. **Centers for Disease Control and Prevention:** Recommendations on prophylaxis and therapy for disseminated *M. avium* complex for adults and adolescents infected with human immunodeficiency virus. Morbidity and Mortality Weekly Report 1993, 42: 17–20.
91. **French GL, Chan CY, Cheung SW, Teoh R, Humphries MJ, O'Mahony G:** Diagnosis of tuberculous meningitis by detection of tuberculostearic acid in cerebrospinal fluid. Lancet 1987, 2: 117–119.
92. **Muranishi H, Nakashima N, Isobe R, Ando T, Shigematsu N:** Measurement of tuberculostearic acid in sputa, pleural effusions, and bronchial washings. Diagnostic Microbiology and Infectious Disease 1990, 13: 235–240.
93. **French GL, Chan CY, Cheung SW, Oo KT:** Diagnosis of pulmonary tuberculosis by detection of tuberculostearic acid in sputum using gas chromatography-mass spectrometry with selected ion monitoring. Journal of Infectious Diseases 1987, 156: 356–362.
94. **Morinet F:** Réaction de polymérisation en chaîne (PCR) en microbiologie. La Presse Médicale 1992, 21: 1377–1380.
95. **Noordhoek GT, Kolk AHJ, Bjune G, Catty D, Dale JW, Fine PEM, Godfrey-Faussett P, Cho S-N, Shinnick T, Svenson SB, Wilson S, van Embden JDA:** Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis:* a blind comparison study among seven laboratories. Journal of Clinical Microbiology 1994, 32: 277–284.
96. **Eisenach KD, Cave MD, Bates JH, Crawford JT:** Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. Journal of Infectious Diseases 1990, 161: 977–981.
97. **Eisenach KD, Sifford MD, Cave MD, Bates JH, Crawford JT:** Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. American Review of Respiratory Disease 1991, 144: 1160–1163.
98. **Brisson-Noël A, Azznar C, Chureau C, Nguyen S, Pierre C, Bartoli M, Bonete R, Pialoux G, Gicquel B, Garrigue G:** Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. Lancet 1991, 338: 364–366.
99. **Clarridge JE, Shawar RM, Shinnick TM, Plikaytis B:** Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. Journal of Clinical Microbiology 1993, 31: 2049–2056.
100. **Fauville-Dufaux M, Vanfleteren B, De Wit L, Vincke JP, Van Vooren JP, Yates MD, Serruys E, Content J:** Rapid identification of tuberculous and non-tuberculous mycobacteria by polymerase chain reaction amplification of a 162 bp DNA fragment from antigen 85. European Journal of Clinical Microbiology and Infectious Diseases 1992, 11: 797–803.
101. **Forbes BA, Hicks KES:** Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. Journal of Clinical Microbiology 1993, 31: 1688–1694.
102. **Shawar RM, El-Zaatari FAK, Nataraj A, Clarridge JE:** Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and nonisotopic hybridization methods. Journal of Clinical Microbiology 1993, 31: 61–65.
103. **Nolte FS, Metchock B, McGowan JE Jr, Edwards O, Okwumabua O, Thurmond C, Mitchell PS, Plikaytis B, Shinnick T:** Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. Journal of Clinical Microbiology 1993, 31: 1777–1782.
104. **Schluger NW, Kinney D, Harkin TJ, Rom WN:** Clinical utility of the polymerase chain reaction in the diagnosis of infections due to *Mycobacterium tuberculosis*. Chest 1994, 105: 1116–1121.

105. **De Wit D, Maartens G, Steyn L:** A comparative study of the polymerase chain reaction and conventional procedures for the diagnosis of tuberculous pleural effusion. Tubercle and Lung Disease 1992, 73: 262–267.
106. **Shankar P, Manjunath N, Mohan KK, Prasad K, Behari M, Ahuja SGK:** Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. Lancet 1991, 337: 5–7.
107. **Noordhoek GT, van Embden JDA, Kolk AHJ:** Questionable reliability of the polymerase chain reaction in the detection of *Mycobacterium tuberculosis*. New England Journal of Medicine 1993, 329: 2036.
108. **Noordhoek GT, Kolk AHJ, Bjune G, Catty D, Dale JW, Fine PEM, Godfrey-Faussett P, Cho S-N, Shinnick T, Svenson SB, Wilson S, Van Embden JDA:** Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. Journal of Clinical Microbiology 1994, 32: 277–284.
109. **Abe C, Hirano K, Wada M, Kazumi Y, Takahashi M, Fukasawa Y, Yoshimura T, Miyagi C, Goto S:** Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe amplified *Mycobacterium tuberculosis* direct test. Journal of Clinical Microbiology 1993, 31: 3270–3274.
110. **Walker GT, Fraiser MS, Schram JL, Little MC, Nadeau JG, Malinowski DP:** Strand displacement amplification – an isothermal, in vitro DNA amplification technique. Nucleic Acids Research 1992, 20: 1691–1696.
111. **Vandervliet GME, Schukkink RAF, Vangemen B, Schepers P, Klatser PR:** Nucleic acid sequence-based amplification (NASBA) for the identification of mycobacteria. Journal of General Microbiology 1993, 139: 2423–2429.
112. **Winn-Deen ES, Batt CA, Wiedman M:** Non-respiratory detection of *Mycobacterium tuberculosis* LCR products in a microtitre plate format. Molecular and Cellular Probes 1993, 7: 179–186.
113. **Wolcott MJ:** Advances in nucleic acid-based detection methods. Clinical Microbiology Reviews 1992, 5: 370–386.
114. **Edwards U, Rogall T, Blöcker HJ, Emde M, Böttger EC:** Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research 1989, 17: 7843–7853.
115. **Kirschner P, Springer B, Vogel U, Meier A, Wrede A, Kieckenbeck M, Bange FC, Böttger EC:** Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. Journal of Clinical Microbiology 1993, 31: 2882–2889.
116. **Plikaytis BB, Plikaytis BD, Yakrus MA, Butler WR, Woodley CL, Silcox VA, Shinnick TS:** Differentiation of slowly growing *Mycobacterium* species, including *Mycobacterium tuberculosis*, by gene amplification and restriction fragment length polymorphism analysis. Journal of Clinical Microbiology 1992, 30: 1815–1822.
117. **Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer T:** Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. Journal of Clinical Microbiology 1993, 31: 175–178.
118. **Lungu O, Della-Latta P, Weitzman I, Silverstein S:** Differentiation of *Nocardia* from rapidly growing *Mycobacterium* species by PCR-RFLP analysis. Diagnostic Microbiology and Infectious Disease 1994, 18: 13–18.
119. **Zhang Y, Heym B, Allen B, Young D, Cole S:** The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature 1992, 358: 591–593.
120. **Zhang Y, Garbe T, Young D:** Transformation with *katG* restores isoniazid-sensitivity in *Mycobacterium tuberculosis* isolates resistant to a range of drug concentrations. Molecular Microbiology 1993, 8: 521–524.
121. **Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T:** Detection of rifampin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 1993, 341–350.
122. **Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T:** Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single-strand conformation polymorphism analysis. Antimicrobial Agents and Chemotherapy 1993, 37: 2054–2058.
123. **Nair J, Rouse DA, Bai G-H, Morris SL:** The rpsL gene and streptomycin resistance in single and multiple drug-resistant strains of *Mycobacterium tuberculosis*. Molecular Microbiology 1993, 10: 521–527.
124. **Meier A, Kirschner P, Bange F-C, Vogel U, Böttger EC:** Genetic alterations in streptomycin-resistant *Mycobacterium tuberculosis*: mapping of mutations conferring resistance. Antimicrobial Agents and Chemotherapy 1994, 38: 228–233.
125. **Honoré N, Cole ST:** Streptomycin resistance in mycobacteria. Antimicrobial Agents and Chemotherapy 1994, 38: 238–242.
126. **Jacobs W, Barletta PR, Udani R, Chan J, Kalkut G, Sosne G, Kieser T, Sarkis G, Hatfull G, Bloom B:** Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. Science 1993, 260: 819–822.
127. **Williams DL, Waguespack C, Eisenach K, Crawford JT, Portaels F, Salfinger M, Nolan CM, Abe C, Sticht-Groh V, Gillis TP:** Characterization of rifampin resistance in pathogenic mycobacteria. Antimicrobial Agents and Chemotherapy 1994, 38: 2380–2386.
128. **Eisenach KD, Crawford JT, Bates JH:** Genetic relatedness among strains of the *Mycobacterium tuberculosis* complex. Analysis of restriction fragment heterogenicity using cloned DNA probes. American Review of Respiratory Disease 1986, 133: 1065–1068.
129. **Eisenach KD, Crawford JT, Bates JH:** Repetitive DNA sequences as probes for *Mycobacterium tuberculosis*. Journal of Clinical Microbiology 1988, 26: 2240–2245.
130. **Van Soolingen D, Hermans PWM, De Haas PEW, Van Embden JDA:** Insertion element IS 1081-associated restriction fragment length polymorphisms in *Mycobacterium tuberculosis* complex species: a reliable tool for recognizing *Mycobacterium bovis* BCG. Journal of Clinical Microbiology 1992, 30: 1772–1777.
131. **Cave MD, Eisenach KD, Templeton G, Salfinger M, Mazurek G, Bates JH, Crawford JT:** Stability of DNA fingerprint patterns produced with IS6110 in strains of *Mycobacterium tuberculosis*. Journal of Clinical Microbiology 1994, 32: 262–266.
132. **Van Embden JDA, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM, Small PM:** Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. Journal of Clinical Microbiology 1993, 31: 406–409.

133. **Dwyer B, Jackson K, Raios K, Sievers A, Wilshire E, Ross B:** DNA restriction fragment analysis to define an extended cluster of tuberculosis in homeless men and their associates. Journal of Infectious Diseases 1993, 167: 490–494.
134. **Jereb JA, Burwen DR, Dooley SW, Haas WH, Crawford JT, Geiter LJ, Edmond MB, Dowling JN, Shapiro R, Pasculle AW, Shanahan SL, Jarvis WR:** Nosocomial outbreak of tuberculosis in a renal transplant unit: application of a new technique for restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates. Journal of Infectious Diseases 1993, 168: 1219–1224.
135. **Haas WH, Butler WR, Woodley CL, Crawford JT:** Mixed-linker polymerase chain reaction: a new method for rapid fingerprinting of isolates of the *Mycobacterium tuberculosis* complex. Journal of Clinical Microbiology 1993, 31: 1293–1298.
136. **Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, Schecter GF, Daley CL, Schoolnik GK:** The epidemiology of tuberculosis in San Francisco. New England Journal of Medicine 1994, 330: 1703–1709.
137. **Small PM, McClenny NB, Singh SP, Schoolnik GK, Tompkins LS, Mickelsen PA:** Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. Journal of Clinical Microbiology 1994, 31: 1677–1682.
138. **Kubica GP, Dye WE:** 1967 clinical and public health: laboratory methods for mycobacteriology. Public Health Service publication no 1547. U.S. Department of Health, Education, and Welfare, Washington DC, 1967.
139. **Hawkins JE, Karlson AG, Wayne L, Wolinsky E:** Quality of laboratory services for mycobacterial disease. American Review of Respiratory Disease 1974, 110: 376–377.
140. **American Thoracic Society:** Levels of laboratory services for mycobacterial diseases: official statement of the American Thoracic Society. American Review of Respiratory Disease 1983, 128: 213.
141. **Hawkins JE, Good RC, Kubica GP, Gangadharam PR, Gruft HM, Stottmeier KD:** The levels of service concept in mycobacteriology. American Thoracic Society News 1983, 9: 19–25.
142. **Clancy L, Rieder HL, Enarson DA, Spinaci S:** Tuberculosis elimination in the countries of Europe and other industrialized countries. European Respiratory Journal 1991, 4: 1288–1295.
143. **Robinson A:** Rationale for cost-effective laboratory medicine. Clinical Microbiology Reviews 1994, 7: 185–199.