Z. Zwolska

MODERN MICROBIOLOGICAL DIAGNOSTIC METHODS OF TUBERCULOSIS IN CLINICAL PRACTICE

National Tuberculosis and Lung Disease research Institute

Mycobacterium tuberculosis is included in Risk Group III in the 1983 WHO classification of risk, along with other microorganisms most likely to infect laboratory workers by airborne route. The number of tubercle bacilli required to initiate infection is low, the infective dose being less than 10 bacilli. **Protecting the laboratory workers** against infection should be the first consideration in mycobacteriology. The recent increase in multidrug-resistant tuberculosis has made safety even more critical to laboratory operation. Most laboratory manipulation of clinical specimens and cultures of microorganisms create aerosols. Although it is impossible to prevent aerosol production and the resultant droplet nuclei, aerosol formation can be minimized, and those aerosols that are produced can be contained. The control of airflow, such as one-pass air conditioning with negative pressure containment laboratories and biological safety cabinets, helps isolate and eliminate any droplet nuclei created from laboratory-generated aerosols. Protective clothing, autoclave sterilization, ultraviolet light, and disinfectant all contribute to protecting laboratory workers and others from infection in the laboratory.

No infected material should leave the laboratory except when it is properly packed for transport to another laboratory. All pathological material, smear, cultures and containers should at least be disinfected and preferably sterilised before disposal or re-use. Autoclaving is the optimal initial sterilisation procedure.

Tuberculosis laboratory services should be organized according to the 3 levels of general health services, i.e: Ist, IInd and IIIth. At the apex of tuberculosis laboratory network should be national reference laboratory (NRL) (one/25—50 mln population is recommended). Its plays an essential role in the organization and maintenance of the network.

The first step in the detection of *M. tuberculosis* and other mycobacteria is the suspicion by the physician who will order **acid-fast microscopy** (AFB) and appropriate cultures. Proper specimen collection and transport are critical to producing a laboratory report. To ensure the best sputum for mycobacteriology, the specimen collector must be trained properly, the patient must be given instructions and must understand them, and the patient must be supervised during sputum collection. An adequate specimen is 5 to 10 ml of sputum. Saliva and nasopharyngeal discharges are not acceptable. Some other specimens that may be submitted to the laboratory for mycobacteria isolation are specimens collected during bronchoscopy, gastric washing, body fluids, tissue, urine, pus drainage, bone marrow, cerebrospinal fluid and others.

Mycobacteria grow slowly, with cell doubling time 15—24 h, depending on growth conditions compared to most other bacteria, which reproduce in 20 min. Therefore, in order to mycobacteria from specimens that contain large numbers of normal flora, **decontamination** of specimens is needed. The rate of contamination should be between 2—5 %; if it is above 5 % then too many mycobacteria are being killed. The one most commonly used in tb laboratories method of digestion and decontamination is the *N*-acetyl-Lcysteine (NALC) — sodium hydroxide (NaOH) method in final concentration of 2 % NaOH for 15—20 min, followed by centrifugation at 3000 x g. The supernatant is carefully decanted, and the pellet is used to prepare smears and inoculate cultured media. The amount of centrifugal force or g's, and the temperature of centrifugation are important variables in the recovery of mycobacteria. The high lipid content of mycobacteria works to keep them buoyant and prevent their concentration if the centrifugal force is too low.

Smear microscopy is the first confirmation of a clinical diagnosis and can be accomplished within one day after collection of specimen. It is enough to have the AFB smear report to diagnose tuberculosis ? Detection of AFB in a sputum smear is not affirmative for *M.tuberculosis* because some nontuberculous mycobacteria (MOTT) also cause pulmonary disease and many of them exist in supply water as a contamination. Positive smear results, if the AFB found are *M.tuberculosis*, indicate that the patient is highly infectious. Negative smear results do not exclude the presents of less than 10.000 organisms/ml in the sputum. So, it is not conclusive for the diagnosis in case of positive findings, and it is not sensitive enough for exclusion of tuberculosis when the results are negative.

Culture isolation is a much more sensitive method than smear examination The routine **cultivation** and detection of mycobacteria have undergone a number of dramatic changes during the last two decades. Traditionally, mycobacteriology has employed both egg-based (Lowenstein-Jensen) and agar-based (Middlebrook 7H10 or 7H110) media. Each medium has advantages and disadvantages, and they compliment each other. The Bactec (Becton Dickinson Diagnostic Systems) radiometric (460-Tb) and fluorometric (960-Tb) systems have advantages of increased recovery, especially when combined with one of the conventional culture media, and decreased time to detection of positive culture an average time 8—14 days. In addition to rapid detection of mycobacteria, the Bactec systems can also be used for **drug susceptibility** testing and the results are usually ready in 5—7 days, compared to 21 days required for conventional testing. Like conventional testing, the Bactec method is also designed to detect the emergence of drug resistance at the 1 % level. To date, the only drugs for which a standardized method is available are the first-line drugs (INH, RMP, SM, EMB), including PZA.

The current "gold standard" for Tb diagnosis is by culture. Culture is about 500 times more sensitive than microscopy and provides organisms for futher investigations including drug sensitivity and genotyping.

A negative culture report on an AFB smear-positive specimen may indicate the presence of nonculivable bacilli. A positive culture report on AFB negative specimen is typical for patients undergoing successful therapy. One of the major problems, recently reported in the literature, is false-positive culture reports due to cross-contamination in the laboratory. But now this problem is easier to recognize by application **RFLP technique**. Strain identification is important when considering control of tuberculosis in the community. The main method for categorising strains is to fingerprint using IS*6110* as a probe. This is useful as it allows characterisation of an outbreak.

The classical **speciation of mycobacteria** include a variety of biochemical tests and cultivation procedures. Recently DNA and RNA probes (Becton-Dickinson and San Diego CA) are available to identify organism in a liquid or solid medium and they are 99—100 % specific. Next new technology is HPLC analysis of mycolic acids. High-performance liquid chromatography has become an integral component of accurate speciation of mycobacteria in many labs. HPLC is highly specific in mycobacterial identification, and it is expected that will replace biochemical methods as a standard for speciation.

In conclusion we should emphasized that the close cooperation between the clinician and the laboratory is essential for high quality management of tuberculosis patients. Barriers in communications are related to either a lack of appreciation of the physician's needs by the laboratory, or insufficient understanding of the procedures the specific for the mycobacteriology laboratory by the physician.

REFERENCES

1. *Starke J. R, Heifets I.*: Navigating through laboratory reports; expectations, dreams, and realities. Seminars in Respir and Critical Care Med. 1997, 18, 5, 509—522.
2. *Kantor I, Kim S J, Frieden T. et al:* Laboratory services in tuberculosis control. Organization and management. Part I WHO/TB /98.258
3. *Huggett J. F., McHugh T. D, Zumla A.:* Tuberculosis: amplification-based clinical diagnostic techniques. Intern J Bioch &Cell Biol. 2003, 35, 1407—1412.
4. *Herold C. D. Fitzgerald R. I, Herold D. A.:* Current techniques in mycobacterial detection and speciation. Critical Rev. Clin Laboratory Science 1996,33(2): 83—138.
5. *Rieder H. L, Chonde T. M., Myking H, et al.:* The public health service national tuberculosis reference laboratory and the national laboratory network. Minimum requirements, role and operation in a low-income country. IUAT&LD1998.
6. *Zwolska Z., Augustynowicz-Kopec E., Klatt M.:* Primary and acquired drug resistance in Polish tuberculosis patients: results of a study of the national drug resistance surveillance programme. Int J Tuberc Lung Dis 2000 4 (9): 832—838.
7. *Sajduda A., Brzostek A., Poplawska M. et al:* Molecular epidemiology of drug-resistant mycobacterium tuberculosis strains isolated from patients with pulmonary tuberculosis in Poland; a 1-year study.

MODERN MICROBIOLOGICAL DIAGNOSTIC METHODS OF TUBERCULOSIS IN CLINICAL PRACTICE

Z. Zwolska

Summary

Tuberculosis bacteriology is one of the fundamental aspects of a national tuberculosis control programmes and a key component of the DOTS strategy. Tuberculosis laboratory service is the most neglected component of these programmes. Mycobacteriology serves in the detection of sources of infection, the diagnosis of clinical suspects, and the follow-up of the effect and results of treatment.

The main purpose of the presentation is to address the problems proper organization of mycobacteriology laboratory including, choice of the methods, algorithm, interpretation of the laboratory reports, modern equipment, application of molecular biology and how to prevent of false-positive results.

Tuberculosis laboratory services cover various activities, which differ from country to country and even from region to region within a country. These can be summarized as follows:

1. detection of acid-fast bacilli by microscopy (AFB); 2. culturing of clinical specimens for mycobacteria; 3. identification of mycobacterial species; 4. performance of drug susceptibility testing (DST); 5. participation in epidemiological research; 6. collection and analysis of laboratory data for epidemiological purposes; 7. consultation with health care workers on the diagnosis and management of tuberculosis; 8. performance of quality assurance and proficiency testing.

Not all of these activities can be carried out by every tb laboratory. The WHO recommends that the full spectrum of mycobacteriologic support be concentrated in only a few laboratories in a given community or region where professional expertise and complete and safe facilities are available.