Role of tuberculosis laboratories in Saudi Arabia

A call to implement standardized procedures

Sahal A. Al-Hajoj, *PhD,* ***Fahad A. Alrabiah,*** *MD.*

ABSTRACT

There is no doubt that the laboratory is the backbone for the diagnosis of tuberculosis (TB). Only through testing in the laboratory can the physician confirm suspicion of TB despite any previous clinical and x-ray findings. Recent visits to several laboratories in the Kingdom of Saudi Arabia showed that some need considerable improvement. Unless there are standardized procedures to diagnose TB, and safety measures are implemented in all laboratories, it will be impossible to diagnose accurately and control TB. The laboratories should be redesigned to conform to international TB Diagnostic Centers, with well trained staff and proper safety procedures.

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Only through testing in the laboratory can the physician confirm his/her suspicion of tuberculosis (TB) despite any previous clinical and x-ray findings. Microscopic examination for the presence of acid fast bacilli (AFB), isolation and recovery of the organism by cultural methods, phenotypic, biochemical, or other contemporary means to identify the recovered organism, and anti-TB susceptibility testing takes place only in the laboratory. In addition, due to the extended growth period of this group of organisms, it is imperative that the laboratory has the means to provide information rapidly to the clinician in case the patient needs to be in isolation and also so that rational therapy, as determined by sensitivity testing, can be implemented promptly. Without these methods, clinical and x-ray findings are merely suspicions that might lead to a false diagnosis.[1-6] On the other hand, false positive results can be generated in the laboratory that may lead to unnecessary treatment (please see cross-contamination paragraph for further details). More than 25 species in the *Mycobacterium* genus are capable of causing human disease. The 5 species most frequently encountered are *Mycobacterium tuberculosis, (M.tuberculosis) M. avium, M. kansasii, M. fortuitum and M. chelonei*.[7-9] Confusion between these species may occur in a laboratory that does not meet the requirements for a TB diagnostic laboratory. Between 1992 and 1995, there was an annual decline of 14.5% in the number of TB cases reported in the United States of America and a 10.3% decline in the State of Pennsylvania alone, over the same period. Among the factors responsible for the decline, the Centers for Disease Control (CDC) credits improved laboratory methods for prompt identification of *M. tuberculosis* and the broader use of drug-susceptibility testing.[10] The importance of the laboratory cannot be over-emphasized. Priority should be given to ensure that technicians have the means to work to achieve the highest possible

From the Department of Comparative Medicine (Al-Hajoj), Department of Medicine (Alrabiah), King Faisal Specialist Hospital and Research Centre, Riyadh, *Kingdom of Saudi Arabia*.

Address correspondence and reprint request to: Dr. Sahal A. Al-Hajoj, King Faisal Specialist Hospital and Research Centre, Riyadh, *Kingdom of Saudi Arabia*. Tel. +966 (1) 4647272 Ext. 32955. Fax. +966 (1) 4647272 Ext. 32962. E-mail: hajoj@kfshrc.edu.sa

Table 1 - MDR-TB profile in different cities within the Kingdom of Saudi Arabia.

City	Drug resistance					MDR-TB (%)	Reference
	RIF	INH	PZA	ETB	STR		
Jeddah	20.8	28.7	7.9	6.9	22.8	25	33,34
Riyadh	2.8	9.1	5	2.8	1.6	11.8	35
Jizan (South)	43	80	S	NA	53	44	36
Dammam	0.2	6	S	S	0.7	7	37

The above table is a summary of studies from different regions showing the percentage of drug resistant TB for single and multi anti-TB agents. It is not clear which method/s was used in each study. RIF - rifampicin, INH - isoniazid, PZA - pyrazinamide, ETB - ethambutol, STR - streptomycin, MDR-TB multi drug resistant tuberculosis

standards with 100% accuracy in an environment that offers them 100% safety.[9,11]

Recent visits to several laboratories in the Kingdom of Saudi Arabia (KSA) showed that some of them need considerable improvement. The purpose of this paper is to discuss the role of TB laboratories, to draw the attention of the authorities to the deficiencies and to call for the implementation of standardized procedures for the diagnosis of TB throughout KSA.

Diagnosis of tuberculosis. Microscopy, culture and identification. The role of the laboratory staff starts with preparing the slides for staining to identify the acid-fast bacilli that will confirm the presence of the *Mycobacterium species (spp.)* in patient samples. Usually, the physician will receive a call from the laboratory to confirm his/her suspicion; however, the work in the laboratory does not end there. The positivity shown on the slide is confirmed by culturing the organism. A growth of bacteria means that the patient definitely has an infection (if cross-contamination is ruled out). Isolation of the bacteria can usually be followed by identification of the species. Is it *M.tuberculosis* or a different species? In addition, the patient's progress is followed by the laboratory reports on the negativity of successive smears and cultures. Some of the laboratories visited did not perform these basic tests for identification nor were sensitivity tests performed on unidentified organisms.[12-14]

Sensitivity tests. Anti-microbial sensitivity tests are performed in the laboratory after isolation and identification of the organism. Different methods are used to test susceptibility to rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide.[15] Among these methods are Bactec 12B, MIGIT-900, and drugs incorporated into solid media. Some laboratories use MIGIT, and others use incorporated drug methods or send their isolates abroad for testing; some laboratories perform no sensitivity tests at all. **Table 1** shows some of the results achieved by different methods used in some cities in KSA. The use of different methods makes comparison of drug resistance difficult. This difficulty is exacerbated when isolates are sent to foreign laboratories where totally different methods are used, or when sensitivity test are not performed or are performed on unidentified organisms.[16-20] It is worth noting that the MIGIT system is still under investigation regarding verification of the second line of anti-TB sensitivity tests despite its use for the first line.

Drug resistance. Some authors discuss different resistance rates in different regions (**Table 1**). There is no doubt that this occurs, and this reinforces the necessity for standardized procedures throughout the country for correct comparison of results.

Cross-contamination. Cross-contamination occurs when bacilli are transferred from a positive specimen to a negative specimen and false positive results will be the outcome. Cross-contamination usually takes place during the processing of specimens in batches either by simple transference from one specimen to another or by aerosols used during treatment and decontamination of the specimens. All specimens processed within one month might be susceptible to cross-contamination. The cost of cross-contamination is high. It is known that the rate of cross-contamination is up to 3% in some of the best laboratories in the world; no laboratory can completely avoid cross-contamination. It is an odd situation where the laboratory has to reach the right diagnosis and at the same time rule out the possibility of cross-contamination which occurs only in the laboratory. A misdiagnosis of TB results in unnecessary treatment and investigations. Medical intervention is costly and can involve risk to the patient. The health care costs of false-positive *M. tuberculosis* cultures can be considerable when Public Health Officials follow up, and when laboratory time and additional screening of family

contacts and hospital employees are taken into account. A diagnosis of TB also can become a psychological burden to patients and their families. It is the serious responsibility of a laboratory to prevent cross-contamination.[21-25] It is not clear how this can be achieved, neither is it clear how cross-contamination can be dealt with when it does take place.

Safety. Safety in the mycobacteriology laboratory is of paramount concern. Sputum samples and other clinical specimens from patients with known or suspected TB must be considered potentially infectious. Aerosols must be controlled by the use of biological safety cabinets with safety carriers. Laboratory staff must strictly follow the safety guidelines.[26-31] Proper safety training should be conducted to ensure the implementation of safety guidelines. These include the following:

Negative pressure rooms. Appropriate ventilation includes negative pressure rooms where the air is filtered through a high-energy particulate air (HEPA) filter and exhausted directly to the outside. 1. Existing facilities maintain a minimum of 6 air exchanges per hour. 2. Newly constructed or renovated facilities maintain a minimum of 12 air exchanges per hour.

Personal safety. 1. Masks worn by staff in TB laboratories must form a tight seal around the face. 2. Face-fit testing must be performed at the time of initial hire of personnel and whenever there is a change in the employee's facial structure (weight loss, surgery, for example.) It is recommended that face fit testing be performed every other year. 3. Laboratory workers must be alert to the signs and symptoms of TB and protect themselves from inadvertent exposure. 4. Tuberculosis skin test should be carried out annually. 5. Employees must understand the risks of TB in their work area. 6. Good infection control must be practiced in the work area.

Exposure control plan review. 1. The plan must be reviewed at least every 2 years and revised as necessary. 2. The purpose of the plan is to protect laboratory workers from exposure to TB.

Recommendations for the standardization of laboratory procedures. 1. Microscopy, culture, identification and sensitivity test. For microscopic examination of specimens, fluorescent staining methods are recommended as they allow faster scanning for acid-fast bacilli (AFB) than conventional Ziehl-Neelsen or Kinyoun methods. This procedure should be available 24 hours a day. Fluorochrome and confirmation with carbol-fuchsin staining should be carried out. Positive results should be reported immediately to the referring physician. Culture remains the definitive way to confirm an infection; non-radiometric methods (such as MIGIT, Becton Dickinson) are recommended since they allow faster detection of *mycobacteria* than growth on conventional solid media.[9,11] However, it is highly recommended that Lenstin-Jonson (LJ) solid media be available as well as liquid cultures. This is to confirm the purity of growth and to extract DNA for finger printing; confirmation of the culture's purity can be achieved by sub-culturing the growth in the liquid media. Identification of species can be achieved by using the BACTEC r-nitro-a-acetylamino-b-hydroxy-propiophenone(NAP) test that is a nucleic acid probe. This method is recommended above conventional biochemical testing. For drug sensitivity testing, non-radiometric methods are recommended, since they can provide results for evaluating first line anti-TB drugs more quickly than conventional testing on solid media. They are safe to use and there is no worry regarding disposal of radioactive materials. Nowadays MIGIT is being used in a large number of laboratories.[9,11]

Finger printing. As mentioned above, cross-contamination occurs in the laboratory. Restriction fragment length polymorphism (RFLP) IS6110 is the method of choice to rule out cross-contamination. However, RFLP IS6110 does not discriminate between isolates that have a very low copy number (<5) of IS6110. Spoligotyping is the method of choice in such cases.[32] There are methods to identify *mycobacterium* complex, *M.avium* and *mycobacteria* other than *M.tuberculosis*. These methods are not the subject of this paper.

In conclusion, tuberculosis is a reemerging disease and a significant health problem in KSA and indeed worldwide. The diagnosis and appropriate treatment of TB are dependent on the prompt response from the microbiology laboratory. In the light of the above information, unless standardized procedures to diagnose TB, and safety procedures are implemented in our laboratories, it will be impossible to diagnose accurately and control TB. The control of TB can only be achieved if all our laboratories are redesigned to a standard level of TB Diagnostic Centers, with well trained staff and proper safety procedures.

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