Asian Pac J Trop Med
2009;2(2):47-53

Original article

Tuberculosis: an experience from *Mycobacterium* smears and culture analysis

Zeehaida M, Siti Asma H, Siti Hawa H, Zaidah AR, Norbanee TH

Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Abstract

Objective: Simple tests like direct smear of the acid fast bacilli (AFB) and *Mycobacterium* culture could assist the diagnosis of tuberculosis. This study is aimed at reviewing the outcome of smears, culture results and contamination rate among specimens requested for AFB smear and *Mycobacterium* culture. **Methods**: Retrospective laboratory data analysis requesting for *Mycobacterium* culture from January 2005 till December 2006 was done in a tertiary teaching hospital of Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. **Results**: Four hundred and sixty seven (36.6%) isolates grew from 1 277 specimens. Of these isolates, 314 (67.2%) grew *Mycobacterium* tuberculosis, 23 (4.9%) grew *Mycobacterium* other than tuberculosis and 38 (8.1%) grew contaminants. Among the *M. tuberculosis* cultures, 165 (52.5%) had growth of more than 100 confluent colonies, whereas 39 cultures (12.4%) had growth of less than 19 colonies. Direct smear for AFB among smear positive cases showed presence of more than 50 bacilli/line in 231 (49.5%) cases and smear negative cases accounted for 63 (13.5%). Among smear positive cases, 291 (94.5%) cultures grew *Mycobacterium* species and another 17 (5.5%) cultures grew contaminants. In smear negative cases, 32 (62.7%) cultures grew *Mycobacterium* species and 19 (37.3%) cultures grew contaminants. **Conclusion**: The results from data analysis of the *Mycobacterium* cultures should be critically utilized in order to review the laboratory performance and to improve its services in the future. Some of the data is also useful to the administrators of the hospital in terms of estimating the risk of occupational hazard faced by the health care workers.

Keywords: Tuberculosis; *Mycobacterium* tuberculosis; Culture; Acid fast bacilli; Smear; Contamination

INTRODUCTION

Tuberculosis (TB) is still prevalent in Malaysia. Its occurrence is in the increasing trend due to a combination of factors such as an excessive influx of immigrants and Human Immunodeficiency Virus (HIV) infection. It had a significant morbidity and mortality. World Health Organization Global TB database in year 2006 had estimated TB prevalence as 125 per 100 000 Malaysian population including HIV positive cases. The incidence for all cases is 103 per 100 000 population per year. The case detection rate for all TB case is estimated at 58%[1].

Symptoms and signs of pulmonary and extrapulmonary tuberculosis are not specific; it may mimic other diseases[2-4]. Simple tests like direct smear of the acid fast bacilli (AFB) and *Mycobacterium* culture could assist the diagnosis in many patients. Other advanced molecular methods which are sophisticated and rather fast in producing the results are available as research or diagnostic tool[5]. However, isolation of *Mycobacterium* from clinical samples by culture still represents the corner stone on which definitive diagnosis of tuberculosis and other mycobacterioses relies[3].

Correspondence to: Dr Zeehaida Mohamed, MD, Master of Pathology, Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.
Tel: +609-7664608. Fax: +609-7653370.
Email: zeehaida@kck.usm.my

Most of the laboratories in the developing world rely on solid media for culture of *Mycobacterium*. At present, mycobacterial culture can be performed on conventional egg based solid medium such as Lowenstein Jensen (LJ) medium, Ogawa medium and commercialized agar based, such as Middle brook 7H10 or 7H11 and liquid media such as Kirschner™ s or Middlebrook 7H9 broth[3].

Growth for *Mycobacterium* takes at least six to eight weeks by conventional culture compared to automated culture system. The prolonged waiting time for culture results sometimes is further complicated by culture contamination. It is define as the presence of non mycobacterial growth (e. g. non acid fast bacteria and fungi) on the cultured media. It can be determined by gross appearance of LJ slant which gave an appearance of a dark blue/green or bleached out/faded LJ slant or it can be determined microscopically by examination of Ziehl-Neelsen stained smear. Contamination rates are determined by dividing the number of slants discarded due to overgrowth by the total number of slants inoculated, for example: 50 patient samples inoculated to 100 LJ slants (2 slants/sample). If six LJ slants overgrew, 6/100 = 6% contamination rate[6].

The acceptable rate of contamination is 5%. There are factors to consider if contamination rate is less than 5%. It could be due to over decontaminating procedure that resulted in killing of too many mycobacteria. If the contamination is greater than 5%, the problem of under-decontamination should be sorted out[6].

The aims of the study: 1. To review the outcome of the smears, culture results and contamination rate among specimens requested for AFB smear and *Mycobacterium* culture. 2. To determine the sensitivity pattern of anti tuberculosis drug against *Mycobacterium tuberculosis* isolated in Hospital Universiti Sains Malaysia. 3. To determine the distribution of positive *Mycobacterium* culture in Hospital Universiti Sains Malaysia.

MATERIALS AND METHOD

Retrospective laboratory data analysis requesting for AFB and *Mycobacterium* culture from January 2005 till December 2006 was done. The study was carried out retrospectively in the Hospital of Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia, a tertiary teaching hospital. The hospital has 700 beds, with two adult intensive care units (ICUs) (medical and neurosurgical), one neonatal ICU, one high dependency unit, 27 medical wards and 11 surgical wards, including two oncology wards. It also has 13 outpatient clinics.

Clinical specimens for AFB smear and *Mycobacterium* cultures were processed in the Medical Microbiology and Parasitology Laboratory. Demographic data such as patients´ gender, age group, clinic/ward distribution of the specimens, AFB smear and culture results, time for the detection of *Mycobacterium* growth and sensitivity pattern of anti tuberculosis drugs were recorded. The AFB was visualized on microscopic examination of Ziehl-Neelsen stained direct smear. The results for AFB smear were graded according to the number of *Mycobacterium* in presence during microscopy. One plus (1 +) is defined as presence of 1 to 50 bacilli/three lines, 2 + is presence of 50 bacilli/ more than one line, 3 + is presence of 10 to 50 bacilli/line and 4 + is presence of more than 50 bacille/line and negative is defined as absence of *Mycobacterium* after examination of three lines.

The *Mycobacterium* was cultured on egg based LJ and Ogawa solid media and incubated for 8 weeks. The media were inspected weekly for evidence of growth. The *Mycobacterium* growths were graded according to the number of colonies seen on egg-based solid agar. One to 19 colonies were reported in its original number, 1 + is defined as presence of 20 to 100 colonies, 2 + is presence of more than 100 colonies but their appearance is discrete, 3 + is presence of more than 100 colonies but their appearance is confluent and negative is defined as no growth or no visible colony seen[7]. Any positive culture of *Mycobacterium* was sent to a National Reference Tuberculosis Center in Sungai Buloh, Kuala Lumpur, Malaysia for confirmation of *Mycobacterium* identity and susceptibility testing of antituberculosis drugs namely isoniazid, streptomycin, rifampicin and ethambuthol.

This study, the resistance towards *Mycobacterium* is defined as follows. Monoresistance is defined as resistance to only the 1 specified drug, Polyresistance is resistance to more or equal to two first-line drugs, but which drugs not specified[8]. Multidrug resistance (MDR) is defined as resistance to at least the two major anti-tuberculosis drugs, isoniazid and

rifampicin with or without other drugs. Extensively drug resistance (XDR) is defined as resistance to at least rifampicin, isoniazid, a second line injectable drug (capreomycin, kanamycin or amikacin) and a fluoroquinolone[8-10].

Data were analyzed using SPSS version 12.0 and presented as frequency and percentage. The association between AFB smear results and outcome of *Mycobacterium* culture was tested by Chi-square test. Mann Whitney test was used to determine the difference of time of growth detected in *Mycobacterium tuberculosis* (MTB) and *Mycobacterium* other than tuberculosis (MOTT) in culture media as the distribution of time for both groups was not normal.

RESULTS

A total of 1 277 specimens received during the study period. Four hundred and sixty seven (36.6%) isolates grew from 1 277 specimens. Of these isolates, 314 (67.2%) grew MTB, 23 (4.9%) grew MOTT and 38 (8.1%) grew contaminants (Table 1). Among the MTB cultures, 165 (52.5%) cultures had growth of plus three (3+) whereas 39 cultures (12.4%) had growth of less than 19 colonies (Table 2). Direct smear for AFB among smear positive cases showed presence of more than 50 bacilli/line in 231 (49.5%) cases and smear negative cases accounted for 63 (13.5%) (Table 3).

Among smear positive cases, 291 (94.5%) cultures grew *Mycobacterium* species and another 17 (5.5%) cultures grew contaminants. In smear negative cases, 32 (62.7%) cultures grew *Mycobacterium* species and 19 (37.3%) cultures grew contaminants (Table 4).

Median (IQR) of time of growth detected in MTB and MOTT in culture media was 24 (28) and 22 (12) days respectively. The difference was not statistically significant by Mann Whitney test (Z = 0.319, P = 0.750) (data not shown).

Most of the *Mycobacterium tuberculosis* isolates were sensitive to anti-tuberculosis drugs; however a few isolates had demonstrated some resistance pattern. Monoresistance is detected in 13 (2.8%) isolates whereas polyresistance to a combination of isoniazide /streptomycin is 4 (0.9%) and multidrug resistance is 4 (0.9%) as shown in Table 5.

Majority of cases with positive cultures were from general medical wards, followed by general outpatient clinic and medical outpatient clinic which represent 38.8%, 16.5% and 15.2% respectively (Table 6).

Table 1 Distribution of the outcomes for *Mycobacterium* cultures (n = 467)

Outcome of the *Mycobacterium* cultures	Frequency (%)
Mycobacterium tuberculosis	314 (67.2)
Mycobacterium other than tuberculosis	23 (4.9)
Contamination	38 (8.1)
No growth	9 (1.9)
Not available	83 (17.8)
Total	467 (100)

Table 2 Distribution of growth for culture of *Mycobacterium tuberculosis* (n = 314)

Results for *Mycobacterium* growth#	Frequency (%)
Number of colonies seen	39 (12.4)
1+	36 (11.5)
2+	71 (22.6)
3+	165 (52.5)
Not available	3 (1.0)
Total	314 (100)

One to 19 colonies were reported in its original number, 1+ is defined as presence of 20 to 100 colonies, 2+ is presence of more than 100 colonies but their appearance is discrete, 3+ is presence of more than 100 colonies but their appearance is confluent, NA = not available.

Table 3 Distribution of the AFB smears results (n = 467)

Results of the AFB smear *	Frequency (%)
1+	117 (25.1)
2+	15 (3.2)
3+	20 (4.3)
4+	231 (49.5)
Negative	63 (13.5)
Not available	21 (4.5)
Total	467 (100)

* 1+ is presence of 1 to 50 bacilli/three lines, 2+ is presence of 50 bacilli/ > one line, 3+ is presence of 10 to 50 bacilli/line and 4+ is presence of more than 50 bacilli/line.

Table 4 Association between the AFB smear results and the outcome of the *Mycobacterium* cultures

		Mycobacterium culture results	
		MTB & MOTT	Contamination
AFB smear-results	Negative	32 (62.7)	19 (37.3)
	Positive	291 (94.5)	17 (5.5)

$\chi^2 = 48.84$, $df = 1$, $P = <0.001$

Table 5 Resistant Pattern of *Mycobacterium tuberculosis* isolates towards anti-tuberculosis drugs ($n = 467$)

Types of resistant	Number (percentage)
Monoresistant	13 (2.8)
Polyresistant	4 (0.9)
Multiple resistant	4 (0.9)
Extended drug resistant	0 (0)

Table 6 Distribution of cases with positive cultures according to hospital setting ($n = 467$)

Hospital setting	Frequency (%)
Wards	
General medical wards	181 (38.8)
High dependency Unit	12 (2.6)
Surgical ward	10 (2.1)
Intensive care unit	10 (2.1)
Pediatric ward	5 (1.1)
Other wards	12 (2.6)
Clinics	
Medical outpatient clinic	71 (15.2)
General outpatient clinic	77(16.5)
Staff clinic	8 (1.7)
Accident & Emergency unit	7 (1.5)
Orthorhinolaryngology clinic	5 (1.1)
Other clinics	6 (1.2)
Not available	63 (13.5)
Total	467 (100)

DISCUSSION

The contamination rate varies among the studies conducted. For example, Hines *et al* in 2006 demonstrated statistically significant contamination rate of 21.7% by using solid media[11]. However, Lee *et al* in 2003 reported a contamination rate of 10.1% by using LJ media for *Mycobacterium* culture[12]. In this study, the contamination rate was 0.03% which is extremely low for a clinical mycobacteriology laboratory. However, according to published guideline by World Health Organization in 1998, 2-3% contamination rate is acceptable in laboratories that received fresh specimens. It is also important to note that a laboratory which experiences no contamination is probably using a method that kills too many of the tubercle bacilli. The usual decontaminant agent used is sodium hydroxide (NaOH) which is toxic, both for contaminants and for tubercle bacilli; therefore, strict adherence to the indicated timing is required. The NaOH procedure is very robust and may kill up to 60% of tubercle bacilli in clinical specimens[13]. The disadvantage of NaOH may contribute to the occurrence of false negative results of *Mycobacterium* culture.

Majority of MTB and MOTT grew from smear positive cases. Some of the culture contamination does arise from these positive cases. Although contamination grew in both cases, it was noted to grow more often in the smear negative cases. Despite the low contamination rate, a few of contaminations in this study were noted among the sputum samples. Some contaminations were also noted from sterile specimens such as cerebrospinal, pericardial and pleural fluid (data not shown). Contamination of sterile samples could be contributed by poor aseptic technique during specimen collection, the contamination of sterile container and culture media used[13]. Thus, it is very important to emphasis on proper collection and handling of specimen from sterile body sites to prevent culture contamination since decontamination procedure is not performed for sterile specimens[7,14]. Further more, only a single specimen usually obtained from the sterile body site due to the involvement of invasive procedure and its complication. The fact is further complicated with

low number of bacilli found in extra pulmonary compared to pulmonary specimen[3, 4, 15].

Occurrence of a positive culture for *Mycobacterium* in this study was 26.4%. If it is correlated with the rate of contamination in our laboratory, the results for positive cultures is lower than expected because of the possibility of over decontamination of the clinical samples. Measures should be taken to overcome the problem by emphasizing on the aspect of over decontamination such as revising the available standard operating procedure used. Majority of the laboratory used NaOH compared to other decontaminant agents and the recommended strength by WHO ranged from 2-4%[13]. In our laboratory, 4% NaOH is used for decontaminating unsterile samples before inoculation on culture media.

The laboratory records must be free from any transcription error, moreover, it must be legible and complete. A few information in this study such as age, smear and culture results was not complete since the some of the data was absent from the records. The data from record of year 2007 was actually far from complete that it could not be included in the current study since they may affect the outcome of statistical analysis. Records with missing data are an important issue since the data is useful to the researcher for research, to the clinician for treatment plan as well as to the administrator.

In a usual practice, some patients with atypical presentation of tuberculosis having AFB smear negative results were discharged home. In certain cases, they were called back for treatment if their culture results were positive. Though the smear negative patients were less infectious than the smear positive, the transmission rate from patient with negative smear was documented by previous study as 17%[16]. This will contribute to the increase in the incidence of tuberculosis in the community. In this study, the significant proportion of patients with smear negative grew *Mycobacterium* in their cultures and the rest had grown contamination. It was also noted that a proportion of contamination was significantly higher in AFB negative compared to the positive smears.

Contamination may, to some extent, delay the decision for commencing antituberculousis drugs since in most cases other specimens are required for culture. However, further studies need to be done to look at the other possible reasons that can confound the culture result. A multicenter study had demonstrated that the recovery and identification are accelerated by automated TB system. The average detection time of *M. tuberculosis* by non-radiometric MB/BacT ALERT 3D System and the radiometric BACTEC 460 system among negative smear samples were 19.9 and 16.8 days respectively as compared to 32.1 days by LJ solid medium[17]. A molecular method for detection of the mycobacterium may accelerate its diagnosis but it is not useful in all TB cases. A meta-analysis had concluded that PCR is not consistently accurate enough to be routinely recommended for the diagnosis of smear-negative pulmonary tuberculosis[18].

In this study, the occurrence of MDR-TB was 0.9% which is lower compared to other studies. However, the resistance pattern of antituberculosis drugs varies among studies. A study conducted by Network of Supranational Reference Laboratories for the WHO/International Union against Tuberculosis and Lung Diseases Global Project on Anti-TB Drug Resistance showed the prevalence of MDR TB was 19.9%, and among the MDR isolates, 9.9% met the criteria for XDR-TB[8]. Another study conducted in Taiwan, 5.7% were MDR-TB, and 0.4% fulfilled the definition of XDR-TB[10]. In California, an analysis of TB case reports submitted to the state TB registry for the period 1993-2006 was published. In the study, pre-XDR TB was defined as TB with resistance to isoniazid and rifampin and either a fluoroquinolone or second-line injectable agent but not both. Among 424 MDR- TB cases with complete drug susceptibility reporting, 4.2% were extensively drug resistant, and 18% were pre-extensively drug resistant. The proportion of pre-XDR TB cases in the study had increased over time, from 7% in 1993 to 32% in 2005[9]. In 2006, the Australian *Mycobacterium* Reference Laboratory Network identified MDR in 2.4% *M. tuberculosis* isolates. No cases of XDR-TB were identified in 2006. However, an on-going review of laboratory data identified one case of XDR-TB in 2004[19].

Hospital of Universiti Sains Malaysia is a tertiary teaching hospital which received referral cases from most of the districts in the state of Kelantan,

Malaysia. The medical and general clinics of this hospital had a waiting area equipped with air conditioned which is connected to a row of examination rooms and a registration area. Clinics with air conditioned facilities may pose a question to the safety of the healthcare workers handling suspected tuberculosis patients since it will increase the risk of hospital acquired infection. In this study, majority of positive *Mycobacterium* cultures were from medical and general clinics which accounted for 15.2% and 16.5% respectively. As noted, majority of the positive cultures are significantly related to positive AFB smear. It is known that between 5 000 and 10 000 tubercle bacilli per milliliter of sputum are required for direct microscopy to be positive and only a proportion of tuberculosis patients harbor large enough numbers of organisms to be detected in this way[13]. Almost 50% of positive TB cases had *Mycobacterium* burden of more than 50 bacilli per line. The probability that a person who is exposed to *M. tuberculosis* will become infected depends primarily on the concentration of infectious droplet nuclei in the air and the duration of exposure to a person with infectious TB disease. The closer the proximity and the longer the duration of exposure, the higher is the risk for being infected. Close contacts defined by Centers for Disease Control and Prevention are persons who share the same air space in a household or other enclosed environment for a prolonged period (days or weeks, not minutes or hours) with pulmonary TB patients. They are at higher risk for exposure to and infection with *M. tuberculosis*[20].

The same issue exists in the high dependency unit, surgical ward and intensive care unit of the hospital which is a closed setting and equipped with central air condition system. These units had a positive cultures ranging from 2.6 to 2.1%. Aerosols created from regular suctioning procedure of ventilated patients and bronchoscopic procedure[21,22] play a role in the transmission of tuberculosis in these setting. The transmission of tuberculosis from non ventilated patients is by active coughing, nebulization procedure[23] and improper handling of the infectious sputum leftover.

Center for Disease Control and Prevention in 2005 had outlined the characteristics of a patient with TB disease that increase the risk for infectiousness (i) presence of cough (ii) cavitation on chest radiograph (iii) positive acid-fast bacilli (AFB) sputum smear result (iv) respiratory tract disease with involvement of the larynx (substantially infectious) (v) respiratory tract disease with involvement of the lung or pleura (exclusively pleural involvement is less infectious) (vi) failure to cover the mouth and nose when coughing (vii) incorrect, lack of, or short duration of anti tuberculosis treatment and (viii) undergoing cough-inducing or aerosol-generating procedures (e.g., bronchoscopy, sputum induction, and administration of aerosolized medications). Whereas environmental factors that increase the risk for probability of transmission of *M. tuberculosis* include (i) exposure to TB in small, enclosed spaces (ii) inadequate local or general ventilation that results in insufficient dilution or removal of infectious droplet nuclei (iii) recirculation of air containing infectious droplet nuclei (iv) inadequate cleaning and disinfection of medical equipment (v) improper procedures for handling specimens[20]. These characteristics should be critically utilized by the health care workers as well as the administrators of the hospital to minimize the risk of occupational hazard in local setting.

In conclusion, the results from data analysis of the *Mycobacterium* cultures should be critically utilized in order to improve the laboratory performance and its services in the future. Some of the data is also useful to the administrators of the hospital in terms of estimating the risk of occupational hazard faced by the health care workers. Where applicable, the necessary corrective action should be planned and acted upon to protect them from occupational disease.

ACKNOWLEDGEMENT

The authors would like to thank all medical technologists from the *Mycobacteriology* Laboratory of the Hospital for their technical assistance.

REFERENCES

1 WHO global TB database and country profile. Available at http://www.who.int/tb/country/global_tb_database/en/index2.html. Last accessed October 23, 2008.

2 **Williams VG**. Tuberculosis: clinical features, diagnosis

and management. *Nurs Stand.* 2006; 20(22):49-53.

3 **Mandell GL**, Bennett JE, Dolin R. Principles and Practices of Infectious Diseases. 5th edition. New York: Churchill Livingstone, 2000.

4 **Bukhary ZA**, Alrajhi AA. Extrapulmonary tuberculosis, clinical presentation and outcome. *Saudi Med J.* 2004; 25(7):881-5.

5 **Negi SS**, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, et al. Diagnostic potential of IS6110, 38kDa, 65kDa and 85B sequence-based polymerase chain reaction in the diagnosis of Mycobacterium tuberculosis in clinical samples. *Indian J Med Microbiol.* 2007; 25(1):43-9.

6 **Ferguson RB**. LJ Inoculation, Reading, and reporting TB cultures. Available at: http://wwwn. cdc. gov/dls/ila/cd/tuberculosisworkshop/files/LJ% ,20 Inoculation,%20 Reading,%20 and %20 Reporting% 20 TB. ppt. Last accessed November 10, 2008.

7 WHO guidelines. Blood safety and clinical technology. guidelines on standard operating procedures for MICROBIOLOGY. Updated 2008 .

8 **Shah NS**, Wright A, Bai G, Barrera L, Boulahbal F, Martín-Casabona N, et al. Worldwide Emergence of Extensively Drug-resistant Tuberculosis. *Emerg Infect Dis.* 2007; 13(3):380-7.

9 **Banerjee R**, Allen J, Westenhouse J, Oh P, Elms W, Desmond E, et al. Extensively Drug-Resistant Tuberculosis in California, 1993-2006. *Clin Infect Dis.* 2008; 47:450-7.

10 **Lai CC**, Tan CK, Huang YT, Chou CH, Hung CC, Yang PC, et al. Extensively drug-resistant mycobacterium tuberculosis during a trend of decreasing drug resistance from 2000 through 2006 at a medical center in Taiwan. *Clin Infect Dis.* 2008; 47:e57-63.

11 **Hines N**, Payeur J B, Hoffman L J. Comparison of the recovery of Mycobacterium bovis isolates using the BACTEC MGIT 960 system, BACTEC 460 system, and Middlebrook7H10 and 7H11 solid media. *J Vet Diagn Invest.* 2006. 18:243-250.

12 **Lee JJ**, Suo J, Lin CB, Wang JD, Lin TY, Tsai YC. Comparative evaluation of the BACTEC MGIT 960 system with solid medium for isolation of mycobacteria. *Int J Tuberc Lung Dis.* 2003; 7(6):569-574.

13 **De Kantor IN**, Kim SJ, Frieden T, Laszlo A, Luelmo F, Norval P, et al. Laboratory services in tuberculosis control culture part iii: For the Global Tuberculosis Programme, World Health Organization, Geneva, Switzerland. Jotto Associati s. a. s, Biella, Italy. 1998.

14 **Grandjean L**, Martin L, Gilman RH, Valencia T, Herrera B, QuinoW, et al. Tuberculosis diagnosis and multidrug resistance testing by direct sputum culture in selective broth without decontamination or centrifugation. *Clin Microbiol.* 2008; 46(7):2339-44.

15 **Kwanjana JH**, Harries AD, Hargreaves NJ, Van Gorkom J, Ringdal T, Salaniponi FML. Sputum-smear examination in patients with extrapulmonary tuberculosis in Malawi. *Trans R Soc Trop Med Hyg.* 2000; 94(4):395-8.

16 **Behr MA**, Warren SA, Salamon H, Hopewell PC, Ponce de Leon A, Daley CL, et al. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. *Lancet.* 1999;353:444-49.

17 **Piersimoni C**, Scarparo C, Callegaro A, Tosi CT, Nista D, Bornigia S, et al. Comparison of MB/BacT ALERT 3D System with Radiometric BACTEC System and Lo¨wenstein-Jensen Medium for Recovery and Identification of Mycobacteria from Clinical Specimens: a Multicenter Study. *J Clin Microbiol.* 2001; 39(2):651-7.

18 **Sarmiento OL**, Weigle KA, Alexander J, Weber DJ, Miller WC. Assessment by meta-analysis of PCR for diagnosis of smear-negative pulmonary tuberculosis. *J Clin Microbiol.* 2003;41(7):3233-40.

19 **Lumb R**, Bastian I, Gilpin C, Jelfs P, Keehner T, Sievers A. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2006 a report of the Australian Mycobacterium Reference Laboratory Network. Commun Dis Intell. 2008; 32(1):12-7.

20 **Jensen PA**, Lambert LA, Iademarco MF, Ridzon R. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. MMWR recommendations and reports, December 30, 2005/54 (RR17); 1-141.

21 **Burman WJ** and Reves RR. Review of false-positive cultures for mycobacterium tuberculosis and recommendations for avoiding unnecessary treatment. *Clin Infect Dis.* 2000; 31:1390-5.

22 **Jasmer RM**, Roemer M, Hamilton J, Bunter J, Braden CR, Shinnick TM, et al. A prospective, multicenter study of laboratory cross-contamination of mycobacterium tuberculosis cultures. *Emerg Infect Dis.* 2002; 8(11):1260-63.

23 **French MV**, Dunlop SG, Wetzler TF. Contamination of sputum induction equipment during patient usage. *Appl Microbiol.* 1971: 899-902.