

Evaluation of direct detection of *Mycobacterium tuberculosis* complex in respiratory and non-respiratory clinical specimens using the Cepheid Gene Xpert® system

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ABSTRACT

الأهداف: تقييم مدى حساسية ودقة اختبار جين اكسبرت ام تي بي / ريفامبين (Xpert MTB/Rif assay) للكشف عن عصيات السل الرئوي ومقارنتها بالطرق التقليدية في العينات التنفسية والغير تنفسية المشتبه إصابتها بالسل الرئوي.

الطريقة: أجريت هذه الدراسة المقطعية في المختبر المركزي العسكري وبنك الدم في مدينة الأمير سلطان العسكرية الطبية بالرياض، المملكة العربية السعودية وذلك خلال الفترة من أكتوبر 2011 إلى يناير 2012م. لقد تم تقييم اختبار جين اكسبرت ام تي بي / ريفامبين للكشف عن عصيات السل الرئوي على 239 عينة (172 عينة تنفسية، و 67 عينة غير تنفسية) من 234 مريض يُشتبه إصابتهم بالسل الرئوي ومقارنتها بالطرق التقليدية للفحص المجهرى وزراعة الخلايا.

النتائج: تمت دراسة 239 عينة وكانت النتائج كالتالي 62 (25.9%) موجبة للسل الرئوي تم عزلها من زراعة الخلايا فيما كانت 59 (24.6%) عينة موجبة للسل الرئوي تم عزلها بواسطة اختبار جين اكسبرت ام تي بي / ريفامبين. وأعطت 3 عينات نتائج سلبية خاطئة. وقد أظهرت نتائج التقييم لهذا الاختبار 95.4% (95%CI: 89-100%) لدرجة الحساسية و 100% (95%CI: 93.6-100%) لدرجة الدقة للعينات الغير تنفسية تصل إلى 94.4% (95%CI: 90.2-98.5) ودرجة الدقة تصل إلى 100% (95%CI: 95.8-100%). وكانت نتائج التقييم الإجمالي جين اكسبرت ام تي بي / ريفامبين مقارنة بالطرق التقليدية للكشف عن عصيات السل الرئوي لجميع العينات تصل إلى 95.2% (95%CI: 87.6-100) لدرجة الحساسية و 100% (95%CI: 92.4-100%) لدرجة الدقة.

خاتمة: أثبتت نتائج الدراسة الحالية مدى فعالية جين اكسبرت ام تي بي / ريفامبين في الكشف السريع عن عصيات السل الرئوي في العينات التنفسية والغير تنفسية بحساسية ودقة عالية خلال ساعتين مقارنة مع طرق الزراعة المعتادة التي تأخذ وقت أطول لعزل عصيات السل الرئوي.

Objective: To compare the sensitivity and specificity of Cepheid Gene Xpert®, MTB/RIF assay for direct detection of *Mycobacterium tuberculosis* complex (MTBC) and rifampin (RIF) resistance with conventional methods in respiratory and non-respiratory clinical specimens.

Methods: We used a cross sectional design to evaluate a diagnostic test at the TB Section of the Division of Microbiology, Central Military Laboratory and Blood

Bank, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia from October 2011 to January 2012. The detection of MTBC and RIF resistance using the Xpert® MTB/RIF assay was assessed in 239 (172 respiratory, and 67 non respiratory) specimens received from 234 patients suspected of TB, and compared with conventional smear microscopy and culture methods.

Results: Out of the 239 specimens investigated, 62 (25.9%) were MTBC positive by culture, while 59 (24.6%) were positive by Xpert® assay. Three samples showed false negative Xpert® results. Compared with the culture, the Xpert® assay achieved 95.4% (95% CI: 89-100%) sensitivity, and 100% (95% CI: 93.6-100%) specificity for respiratory samples, while the sensitivity for non-respiratory specimens was 94.4% (95% CI: 90.2-98.5), and the specificity for non-respiratory specimens was 100% (95% CI: 95.8-100%). Overall, a 95.2% (95% CI: 87.6-100) sensitivity, and 100% (95% CI: 92.4-100%) specificity, was observed for the Xpert® MTB/RIF assay compared with conventional methods for MTBC detection.

Conclusion: The gene Xpert® MTB/RIF assay is a helpful tool for the detection of MTBC and RIF resistance in respiratory and non-respiratory clinical samples with a high sensitivity and specificity within 2 hours as compared with conventional methods, which took a much longer time.

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Tuberculosis (TB) is one of the most deadly and common major infectious diseases in developing and industrialized countries, and according to the World Health Organization (WHO), one third of the global population is infected with TB, and approximately 7-8 million new cases of TB occur each year. In 2010, there were an estimated 14 million cases with 1.2-1.7 million deaths (including deaths from TB among HIV-positive people).¹ Tuberculosis (both pulmonary and extra-pulmonary) is also endemic and a major health problem in Saudi Arabia. According to 2011 statistics, 18/100,000 population are infected with TB in this country.² The conventional methods used in the laboratory require microscopy, culture, and drug susceptibility testing (DST), which is a laborious as well as time consuming process.^{3,4} In the case of extra-pulmonary specimens it becomes more difficult to establish *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (MTBC) due to the presence of a lower number of bacteria compared with pulmonary specimens. In addition the collection of extra-pulmonary samples requires invasive procedures, and it is not easy to obtain extra specimens.⁵ Therefore, several new techniques have developed involving nucleic acid amplification to obtain MTB diagnosis more rapidly with high sensitivity and specificity,^{6,7} including the Cepheid Gene Xpert® system (GX) (Cepheid, Maurens-Scopont, France). The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is a hemi-nested real time polymerase chain reaction (RT-PCR) assay for detection of MTBC as well as resistance to rifampin (RIF) by the RIF resistance-determining region of the *rpoB* gene with molecular beacons within 2 hours.⁷ Furthermore, it reduces the infection risk to working persons, and prevents cross contamination between the clinical specimens due to use of a disposable closed cartridge.^{8,9} The aim of this study was to evaluate the performance of the Cepheid Gene Xpert® MTB/RIF assay for direct detection of MTBC and RIF-resistance in respiratory and non-respiratory clinical specimens, and to compare results obtained with the conventional microscopy and culture method.

Methods. This study was conducted in the TB Section of the Division of Microbiology, Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City (PSMMC), Riyadh, Kingdom of Saudi Arabia from October 2011 to January 2012 in order to assess the performance of the Xpert® MTB/RIF assay for direct detection of MTBC and RIF-resistance in respiratory and non-respiratory specimens. All clinically suspected TB samples received in the

TB section during this period were included in the this study. Out of total 239 specimens received from 234 (126 male and 108 female) patients, 172 were respiratory and 67 were non-respiratory specimens. The respiratory specimens includes 56 sputum and 116 broncho-alveolar lavage (BAL) samples, while non-respiratory samples includes 16 tissues, 14 CSF, 5 fine needle aspirates, 10 aspirate from abscesses, 13 pleural fluids (PLFL), 3 pericardial fluids (PCFL), 2 synovial fluids (SYNFL) and 4 abdominal aspirates.

Both respiratory and non-respiratory specimens including tissues and biopsies were processed by the standard N-acetyl-L-cysteine and sodium hydroxide method. Tissues and biopsies were at first, grinded firmly with a small amount of sterile saline by a tissue grinder (Sigma-Aldrich CO Ltd, Taufkircher, Germany), and then processed like other specimens. Freshly prepared Mycoprep™ NALC-NaOH solution (Becton Dickinson Company, Sparks, MD, USA) was added to the specimen at equal volume, mixed on vortex, and left for 15 minutes for digestion at room temperature. A double amount (twice the amount of mixture) of sterile phosphate buffer (pH 6.8) (Becton Dickinson Company, Sparks, MD, USA) was then added to the mixture and centrifuged for 20 minutes at 3000 rpm. The supernatant was removed and the sediment was dissolved in 2.5 ml of sterile phosphate buffer for further study.⁴

Lowenstein-Jensen medium (LJ) (Saudi Prepared Media Laboratories, Riyadh, KSA) was inoculated with 0.5 ml of dissolved specimen solution. The inoculated LJ medium was then incubated at 37°C for 8 weeks and examined weekly, while 0.5 ml of the specimen solution was also added to liquid medium in *Mycobacterium* Growth Indicator Tubes (MGIT) (Becton Dickinson and Company, Sparks, MD, USA). The MGIT tubes were then incubated in an automated MGIT 960 system™ (Becton Dickinson Company, Sparks, MD, USA) at 37°C for 6 weeks.⁵

The remaining deposit was used for PCR investigation using the Xpert® MTB/RIF™ assay (Cepheid, Maurens-Scopont, France). One ml of the remaining deposit of clinical sample was transferred to screw-capped tube containing 2 ml of sample reagent (SR) at a ratio of 1:2, this reagent inactivates the sample with NaOH and isopropanol. The mixture was then incubated for 15

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Table 1 - *Mycobacterium tuberculosis* complex detection by Xpert® MTB/RIF assay (Cepheid, Maurens-Scopont, France) assay.

Specimen types, n	Smear results	Xpert assay results	Detection MTBC by culture	
			Positive	Negative
Sputum, 56	Positive	+	31	0
		-	0	0
	Negative	+	6	0
		-	19	18
Bronchial alveolar lavage, 116	positive	+	4	0
		-	1 [†]	1
		+	1	0
	Negative	-	110	109
		+	2	0
		-	0	0
Tissues, 16	positive	+	6	0
		-	8	7
		+	0	0
Cerebrospinal fluids, 14	Negative	-	0	0
		+	0	0
		-	14	14
Fine needle aspirate, 5	Positive	+	2	0
		-	1	0
		+	0	0
	Negative	-	2	2
		+	0	0
		-	0	0
Body fluid, 22	positive	+	0	0
		-	0	0
		+	3	0
	Negative	-	19	19
		+	2	0
		-	0	0
Abscess, 10	positive	+	2	0
		-	0	0
		+	1	0
	Negative	-	7	7

*False negative Xpert assay result, [†]*Mycobacterium* spp. other than MTBC, MTBC - *Mycobacterium tuberculosis* complex

minutes at room temperature and was mixed every 5 minutes until liquefied with no visible clumps.⁹

The mixture was transferred into the Xpert® MTB/RIF cartridge using the sterile pipette provided until the meniscus was above the minimum mark. The Xpert® MTB/RIF cartridge includes an internal control for sample processing (DNA extraction and for PCR presence inhibitors), afterwards the inoculated cartridge was placed into the Gene Xpert® instrument (GX). Results were available in less than 2 hours and interpreted by the GX System automatically as follows; Positive or negative results were related to the presence or absence of MTB DNA while false results were due to the presence of PCR inhibitors.

While the sterile samples were processed directly without decontamination and digestion, all specimens were centrifuged at 3000 rpm for 20 minutes. The

supernatant discarded and the sediment re-suspended with 2.5 ml of sterile phosphate buffer and used for AFB microscopy, culture and Xpert® PCR as mentioned above. Smear examination for presence of AFB was carried out routinely for all specimens. Smears were prepared, fixed, and stained with auramine-rhodamine stain (REMEL Inc., Lenexa, Kansas, USA) then visualized under an Olympus Fluorescence Microscope (Olympus, New York, USA) at 400 magnifications. The suspected positive slides were confirmed by Ziehl-Neelsen stain (REMEL Inc., Lenexa, Kansas, USA) and the results were recorded according to Centers for Disease Control (CDC) standards.

Positive cultures of *Mycobacterium* isolate were investigated by smear examination, pigment production, and biochemical tests such as nitrate reduction, and Niacin accumulation tests (REMEL Inc, Lenexa, Kansas, USA). The P-nitrobenzoic acid (PNB) (ICN Bio-Medicals Inc, New York, USA) was also used to differentiate the MTBC from other *Mycobacterium* spp. The PNB (250 and 500 µg/ml) was added to the MGIT medium and incubated in an automated MGIT 960 system™ at 37°C for result interpretation.¹⁰ Also, the DST was performed by BACTEC™ MGIT 960 SIRE Kit (Becton Dickinson Diagnostic, Sparks, MD, USA).

Statistical analysis. The data were analyzed statistically using the Binomial Proportion Confidence Interval Method (Excel Software).¹¹

The study was conducted after approval from the Research Ethical Committee of the Prince Sultan Military Medical City, Riyadh, KSA.

Results. A total number of 239 specimens (172 respiratory and 67 non-respiratory) from 234 (126 male and 108 female) patients with suspected TB infection including one positive patient with HIV were assayed for TB detection by Xpert® PCR and conventional methods (Table 1).

A confirmed positive culture of MTBC was used as a reference standard. Out of 239 specimens, 62 (25.9%) were positive for MTBC by culture (MGIT and/or LJ media), 44 (25.5%) respiratory, and 18 (26.8%) non-respiratory specimens. Among 62 positive samples; 41 were smear positive for AFB (35 respiratory and 6 non-respiratory). One positive smear for AFB was negative by Xpert® assay because this was non-tuberculous *Mycobacterium*. Three samples, one each from sputum, BAL and tissue showed false negative Xpert® assay results. Moreover, MTBC resistant to RIF was correctly detected by Xpert® assay in 2 lymph node tissues from different patients.

In the case of respiratory samples the sensitivity of smear was 79.5% (95% confidence interval [CI]: 74.2-84.7%) and specificity was 99.2% (95% CI:93.9-100%) compared with culture results, while non-respiratory specimens achieved 33.3% (95% CI:31.8-34.7.6%) sensitivity, and 100% (95% CI:98.5-100%) specificity (Table 2). In general, compared with culture, the sensitivity for total smear examination was 66.1% (95% CI:60.8%-71.3%) and specificity was 99.4% (95% CI:94.1-100%).

On the other hand, the Xpert® assay detected MTBC in 59 (24.6%) investigated samples (42 respiratory and 17 non-respiratory specimens). In the case of respiratory specimens, the sensitivity and specificity of the Xpert® MTB/RIF assay were 95.4% (95% CI:89-100%) and 100% (95% CI:93.6-100%) while sensitivity and specificity for non-respiratory specimens were 94.4% (95% CI:90.2-98.5%) and 100% (95% CI:95.8-100%) (Table 2). Overall, the sensitivity was 95.2% (95% CI:87.6-100%) and specificity was 100% (95% CI:92.4-100%) of Xpert® MTB/RIF assay compared to the cultures (Table 2). Five isolates of non-tuberculous *Mycobacterium* were not detected by Xpert® MTB/RIF assay and were positive in culture for non-MTBC. No false positive Xpert® assay result was recorded in the study.

Discussion. Conventional methods for detection of MTBC in clinical specimens like microscopy have low sensitivity. The cultures are time consuming, require expertise and laboratory facilities including bio-safety measures to limit the risk to laboratory workers.^{4,5} Molecular techniques have changed the field of TB with rapid diagnosis combined with high sensitivity

and specificity results.⁵⁻⁷ The Cepheid Gene Xpert® system is a molecular based bench top instrument fully automated and integrating including specimens' preparation, amplification, and detection.

The Xpert® MTB/RIF assay is a novel molecular TB-specific, semi-quantitative RT-PCR test, and detects both MTBC and RIF resistance in a single test within 2 hours. This reduces the infection risk to working person and prevents cross contamination between the clinical specimens due to use of disposable closed cartridges.^{12,13}

Many studies have assessed the molecular techniques for detection of MTBC, and the results indicated that the PCR is a useful approach for rapid diagnosis of TB from clinical specimens. However, there are limitations to these techniques including; the sensitivity of the PCR result is mostly dependent on the efficiency of nucleic acid extraction procedures, and presence of PCR inhibitors in some of the clinical specimens.¹⁴ Compared with culture results, smear microscopy for respiratory specimens achieved a 79.5% (95% CI:74.2-84.7) sensitivity, and 99.2% (95% CI:93.9-100%) specificity, whereas the sensitivity of Xpert® MTB/RIF assay was 95.4% (95% CI:89-100%) and the specificity was 100% (95% CI:93.6-100%). These results agreed with a previous study.¹⁵

In the case of non-respiratory specimens, the sensitivity of the Xpert® assay compared with culture was 94.4% (95% CI:90.2-98.5%), with specificity of 100% (95% CI:95.8-100%). These results were found to be higher than previous studies conducted in Germany and France with a sensitivity of 77.3-85%, and 98% specificity,^{9,15} which may be due to specimens received from a particular community. This may also be attributed to higher volume of concentrated sample,

Table 2 - The sensitivity and specificity of the Xpert MTB/RIF assay compared with the culture method in a study conducted in the TB Section of the Division of Microbiology, Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia.

Specimen type	Number of total samples	Method	Sensitivity	Specificity	PPV	NPV
				%(95% CI)		
Respiratory	172	Smear	79.5 (74.2-84.7)	99.2 (93.9 -100)	97.2 (91.9-100)	93.4 (88.1-98.6)
		Xpert MTB/ RIF	95.4 (89-100)	100 (93.6-100)	100 (93.6-100)	98.5 (92.1-100)
Non- respiratory	67	Smear	33.3 (31.8-34.7)	100 (98.5-100)	100 (98.5-100)	80 (78.5-81.4)
		Xpert MTB/ RIF	94.4 (90.2-98.5)	100 (95.8-100)	100(85.8-100)	98 (93.8-100)
Total	239	Smear	66.1 (60.8-71.3)	99.4 (94.1-100)	97.6 (92.3-100)	89.3 (84.0-94.5)
		Xpert MTB/ RIF	95.2 (87.6-100)	100 (92.4-100)	100 (92.4-100)	98.3 (90.7-100)

CI - confidence interval, PPV - positive predictive value, NPV - negative predictive value

more efficient DNA extraction procedures, and a higher incidence of TB compared with other populations. The performance of the Xpert® assay here suggests that it is also a useful tool for rapid detection of extra-pulmonary TB also.^{9,15}

False negative Xpert® PCR results were detected in 3 specimens, 2 respiratory (sputum and BAL) and one non-respiratory (tissue). The possible explanation is the presence of PCR inhibitors or insufficient nucleic acid material in these specimens.¹⁴ These false negative PCR results decreased the sensitivity of the Xpert® assay in this study. In addition, one positive smear for AFB in BAL from an HIV patient was negative for Xpert® assay and positive for non-tuberculous *Mycobacterium* identified as *Mycobacterium chelonae*.

Furthermore, the Xpert® MTB/RIF assay correctly detected the rpo B gene mutation associated with RIF-resistance in 2 samples of lymph nodes. Therefore, the sensitivity and specificity of the Xpert® MTB/RIF assay for the detection of RIF-resistant *M. tuberculosis* were 100%. Utilizing the Xpert® MTB/RIF assay for both respiratory and non-respiratory samples, the sensitivity was 95.2% (95% CI: 87.6-100%), and the specificity was 100% (95% CI: 92.4-100%). These results are slightly higher compared with previous studies.^{15,16} The possible explanation for higher sensitivity and specificity of the Xpert® assay in this study is that the population may have a higher incidence of TB. Several studies recommended using of the Xpert® MTB/RIF assay as the preliminary diagnostic analysis in specimens suspected of having MDR-TB or in HIV associated TB in endemic countries.^{17,18}

The relatively high cost of the Xpert® assay is an important issue that TB control programs should consider. However, the rapid detection of MTBC, simplicity with safety, prevention of cross infection and epidemiological advantage should be weighed against the cost.¹⁹ The Xpert® MTB/RIF contains specific MTBC probes, and so cannot replace culture for *Mycobacterium spp.* Although the current formula includes detection of the RIF resistance gene (rpo B gene), the test is unable to detect other resistance mechanisms, or resistance to other antibiotics, and so the Xpert® MTB/RIF cannot replace conventional culture and DST. Furthermore, conventional methods including smear microscopy and culture are important to rule out other *Mycobacterium species*, and for monitoring treatment response to assist with patient management.

The limitation of this study was the small number of clinical specimens, particularly the non respiratory samples. Further studies with a large number of

non-respiratory specimens, in comparison with histopathology and conventional method will be useful for assessment of the ability of the Xpert® MTB/RIF assay in the diagnosis of extra-pulmonary TB.

In conclusion, our findings demonstrate that the Xpert® assay is a useful tool for the detection of MTBC and RIF resistance with high sensitivity and specificity in respiratory and non-respiratory clinical specimens compared with conventional methods for MTBC isolation.

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References

1. World Health Organization. Global tuberculosis control, 2009. Epidemiology, strategy, financing. Geneva (Switzerland); WHO; 2009. [Accessed 2012 September 15]. Available from URL: http://whqlibdoc.who.int/publications/2009/9789241563802_eng_doc.pdf
2. World Health Organization. Global Tuberculosis Control Report 2010. Geneva (Switzerland); 2010. [Accessed 2012 April 15 2012]. Available from URL: http://www.who.int/tb/features_archive/global_report2010_launch_11nov10/en/index.html
3. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. *Curr Opin Infect Dis* 2009; 22: 174-182.
4. Chakravorty S, Sen MK, Tyagi JS. Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology. *J Clin Microbiol* 2005; 43: 4357-4362.
5. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR Morb Mortal Wkly Rep* 2009; 58: 7-10.
6. Greco S, Girardi E, Navarra A, Saltini C. Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. *Thorax* 2006; 61: 783-790.
7. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; 363: 1005-1015.
8. Banada PP, Sivasubramani SK, Blakemore R, Boehme C, Perkins MD, Fennelly K, et al. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. *J Clin Microbiol* 2010; 48: 3551-3557.
9. Hillemann D, Rüscher-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *J Clin Microbiol* 2011; 49: 1202-1205.
10. Sharma B, Pal N, Malhotra B, Vyas L. Evaluation of a Rapid Differentiation Test for *Mycobacterium Tuberculosis* from other Mycobacteria by Selective Inhibition with p-nitrobenzoic Acid using MGIT 960. *J Lab Physicians* 2010; 2: 89-92.
11. Brown LD, Cai TT, DasGupta A. Interval estimation of binomial proportion. *Stat Sci* 2001; 16: 101-133.

12. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J Clin Microbiol* 2010; 48: 2495-2501.
13. Moore DF, Guzman JA, Mikhail LT. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. *Diagn Microbiol Infect Dis* 2005; 52: 247-254.
14. Honoré-Bouakline S, Vincensini JP, Giacuzzo V, Lagrange PH, Herrmann JL. Rapid diagnosis of extrapulmonary tuberculosis by PCR: impact of sample preparation and DNA extraction. *J Clin Microbiol* 2003; 41: 2323-2329.
15. Malbruny B, Le Marrec G, Courageux K, Leclercq R, Cattoir V. Rapid and efficient detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory samples. *Int J Tuberc Lung Dis* 2011; 15: 553-555.
16. Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaitre N. Comparison of the Xpert MTB/RIF Test with an IS6110-TaqMan Real-Time PCR Assay for direct detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory specimens. *J Clin Microbiol* 2011; 49: 1772-1776.
17. Aljohaney A, Amjadi K, Alvarez GG. A systematic review of the epidemiology, immunopathogenesis, diagnosis, and treatment of pleural TB in HIV-infected patients. *Clin Dev Immunol* 2012; 2012: 842045.
18. Lawn SD, Brooks SV, Kranzer K, Nicol MP, Whitelaw A, Vogt M, et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. *PLoS Med* 2011; 8: e1001067.
19. Al-Jahdali H. For better control, tuberculosis deserves better attention. *Ann Thorac Med* 2012; 7: 1-2.

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Abdel-Aziz NA, Al-Harbi KM, Morsy ME, Turkistani KA. Evaluation of direct detection of *Mycobacterium tuberculosis* in clinical samples using the BD ProbeTec ET system. *Saudi Med J* 2011; 32: 123-127.

Sarhan MA. Tuberculosis vaccine. *Saudi Med J* 2010; 31: 9-13.

Al-Hajoj SA. Molecular strain typing of *Mycobacterium tuberculosis* isolates to detect cross-contamination events. *Proposed modifications to prevent its recurrence*. *Saudi Med J* 2009; 30: 1515-1519.