

ARTÍCULO ORIGINAL

## Xpert MTB/RIF test performance assay in respiratory samples at real work settings in a developing country

Santiago Atehortúa<sup>1</sup>, Faiver Ramírez<sup>2</sup>, Lina María Echeverri<sup>1</sup>, Adrián Peñata<sup>1</sup>, Sigifredo Ospina<sup>1</sup>

<sup>1</sup> Departamento de Microbiología, Hospital Universitario San Vicente Fundación, Medellín, Colombia

<sup>2</sup> Departamento de Enfermedades Infecciosas, IPS Universitaria León XIII, Medellín, Colombia

**Site where the research was conducted:** Hospital Universitario San Vicente Fundación, Medellín, Colombia

**Introduction:** The Xpert MTB/RIF test detects DNA from *Mycobacterium tuberculosis* complex and susceptibility to rifampin. It has been evaluated repeatedly under “ideal” conditions including centrifugation of sputum and bronchoalveolar lavage, Ziehl Neelsen (ZN) and auramine/rhodamine staining, as well as with solid and liquid automated culture methods. Results from such evaluations cannot be extrapolated to low-income countries that do not routinely use all these processes.

**Objective:** To assess the performance of the Xpert MTB/RIF test in respiratory samples under “real” conditions of work in a low-income country and its correlation with phenotypic susceptibility testing.

**Materials and methods:** We conducted a cross-sectional study to assess the performance of the Xpert MTB/RIF test in  $\geq 12$  year-old patients with suspected pulmonary tuberculosis. In routine sample processing at the Hospital we do not use sputum centrifugation, staining with auramine/rhodamine or automated liquid culture.

**Results:** We screened 152 patients of whom 108 were eligible for the study and 103 were included in the analysis; 34% of the samples were positive. The overall test sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 91%, 92%, 83% and 96%, respectively. In ZN-negative samples the sensitivity, specificity, PPV and NPV were 87%, 91%, 68% and 97%, respectively. The results of sensitivity and resistance to rifampin were concordant with susceptibility testing using the multiple proportions method ( $\kappa=1$ ,  $p<0.0001$ ).

**Conclusions:** The Xpert MTB/RIF test overall performance was similar to the one achieved under ideal conditions. Its performance in ZN-negative samples was better under “real” conditions of work in a low-income country.

**Key words:** *Mycobacterium tuberculosis*, tuberculosis, polymerase chain reaction, rifampin.

doi: <http://dx.doi.org/10.7705/biomedica.v35i1.2330>

### Rendimiento de la prueba Xpert MTB/RIF en muestras respiratorias en el escenario real de trabajo en un país en desarrollo

**Introducción.** La prueba Xpert MTB/RIF detecta el ADN del complejo *Mycobacterium tuberculosis* y la sensibilidad a rifampicina. La prueba ha sido evaluada en condiciones “ideales” que incluyen la centrifugación de esputo y el lavado broncoalveolar, la tinción de Ziehl Neelsen (ZN) y de auramina-rodamina y los métodos de cultivo sólido y de cultivo líquido automatizado. Los resultados de tales evaluaciones no pueden extrapolarse a países de bajos ingresos que no utilizan habitualmente todos estos procesos.

**Objetivo.** Evaluar el rendimiento de la prueba Xpert MTB/RIF en muestras respiratorias bajo condiciones “reales” de trabajo y su correlación con las pruebas fenotípicas de sensibilidad.

**Materiales y métodos.** Se llevó a cabo un estudio transversal para evaluar el rendimiento de la prueba Xpert MTB/RIF en pacientes  $\geq 12$  años con sospecha de tuberculosis pulmonar. En el procesamiento rutinario de muestras en el Hospital del estudio no se usa la centrifugación del esputo, la tinción con auramina-rodamina ni el cultivo líquido automatizado.

**Resultados.** Se incluyeron 152 pacientes, de los cuales 108 eran elegibles y 103 se incluyeron en el análisis. El 34 % de las muestras fueron positivas; la sensibilidad de la prueba fue de 91 %, la especificidad de 92 %, el valor diagnóstico positivo de 83 % y el valor diagnóstico negativo global de 96 %. En las muestras negativas con Ziehl Neelsen, la sensibilidad fue de 87 %, la especificidad

#### Author's contributions:

The above authors fully participated in the completion of the protocol, data collection, analysis of results and discussion. Additionally, Santiago Atehortúa and Adrian Peñata participated in the processing of samples.

de 91 % y los valores diagnósticos positivo y negativo alcanzaron 68 y 97 %, respectivamente. Los resultados de sensibilidad o resistencia a la rifampicina concordaron con los de la prueba fenotípica de sensibilidad (valor de kappa=1,  $p<0,0001$ ).

**Conclusiones.** El rendimiento global de la prueba fue similar al obtenido bajo condiciones “ideales”. En las muestras negativas con Ziehl Neelsen se obtuvo un mejor rendimiento en las condiciones “reales” de trabajo de un país de bajos ingresos.

**Palabras clave:** *Mycobacterium tuberculosis*, tuberculosis, reacción en cadena de la polimerasa, rifampicina.

doi: <http://dx.doi.org/10.7705/biomedica.v35i1.2330>

Among infectious diseases, tuberculosis is a major cause of morbidity and mortality in the world and it is considered a public health problem mainly in developing countries. In 2011, 8.7 million people contracted the disease and 1.4 million died (1).

Historically, bacilloscopy and culture of respiratory samples have been used for the diagnosis of pulmonary tuberculosis. Bacilloscopy has a low diagnostic performance (sensitivity, 40-60%), and although culture is more sensitive, 4 to 8 weeks are required for bacilli isolation in solid media, and 2 to 4 weeks in automated liquid cultures, which is enough time for the propagation of sensitive and multidrug resistant (MDR) bacteria, posing significant challenges for the control of the disease (2-5).

Polymerase chain reaction (PCR) has revolutionized microbiology by facilitating direct detection and identification of infectious agents in clinical specimens in a short time (4). The recently introduced Xpert MTB/RIF® test (Cepheid, Inc., Sunnyvale, California, USA) detects the presence of *Mycobacterium tuberculosis* DNA, as well as susceptibility to rifampin (RIF) in real time by recognizing *rpoB* gene mutations in a single reaction. Monoresistance to rifampin is rare, and it is estimated that over 90% of rifampin-resistant strains also exhibit some degree of resistance to isoniazid (INH); thus, detecting RIF resistance can be useful as an MDR tuberculosis marker. This molecular test can reduce to two hours the time it takes to have results from conventional phenotypic sensitivity tests to antimicrobial agents (method of multiple proportions) (5-7).

The Xpert MTB/RIF® test has been evaluated repeatedly under ideal conditions including sputum and bronchoalveolar lavage processed with

centrifugation, ZN and auramine/rhodamine staining, as well as solid culture and liquid automated culture. The results of such evaluations cannot be extrapolated to many low-income countries that do not use these processes routinely (6,8).

The aim of this study was to assess the performance of the Xpert MTB/RIF® test for the diagnosis of pulmonary tuberculosis in respiratory samples compared with culture and its correlation with phenotypic susceptibility testing in “real” working conditions in a developing country hospital with high prevalence of the disease and limited resources (9).

The study was conducted in the city of Medellín, Colombia, where tuberculosis incidence amounts to 78.9 cases per 100,000 population as reported by the bulletin of the *Dirección Seccional de Antioquia*, 2012.

## Materials and methods

### Study settings

A cross-sectional study was conducted in order to evaluate the Xpert MTB/RIF diagnostic test accuracy in identifying pulmonary tuberculosis disease in patients from the *Hospital Universitario San Vicente Fundación* in Medellín, Colombia, from September 2010 to December 2011. The Hospital Ethics Committee approved the study.

### Study participants

Participants were chosen from among  $\geq 12$  year-old patients with clinical suspicion of pulmonary tuberculosis. Clinical suspicion of tuberculosis was defined in HIV-negative patients as cough for 14 or more days and in HIV-positive patients as cough during any period of time, while as well as in other patients with concurrent abnormal chest x-ray (cavity, focal opacity, pleural effusion or nodule).

### Sample collection and processing

The respiratory samples analyzed were sputum, bronchoalveolar lavage or tracheal aspirate (one sample per patient). All samples were stained with Ziehl-Neelsen (ZN), inoculated in Ogawa-Kudoh

Corresponding author:  
Faiver Ramírez, Clínica León XIII, Calle 69 N° 51C-24, bloque 2,  
Medellín, Colombia  
Telephone: (574) 250 9284; fax: (574) 233 7316  
[faiverramirezbrinez@gmail.com](mailto:faiverramirezbrinez@gmail.com)

Recibido: 09/03/14; aceptado: 10/12/14

(OK) culture and tested with the Xpert MTB/RIF® assay. Samples were processed following the hospital protocol and the test manufacturer's recommendations.

Bronchoalveolar lavage was centrifuged at 3,500 gravities for 30 minutes in refrigerated centrifuge (Hettich®), and the supernatant was discarded leaving 2 ml of sediment; 1 ml was taken for standard tests (ZN stain and OK culture) and 1 ml for molecular testing. Sputum and tracheal aspirate did not undergo centrifugation.

Microbiological culture samples were decontaminated with sodium hydroxide (NaOH) 4% and cultured using the Kudoh swab method (direct method) in two tubes with Ogawa-Kudoh medium; liquid medium was not used. The culture was incubated at 37°C in horizontal position for 8 weeks and reading was performed each week; after the eighth week, if no growth was seen, it was reported as negative.

**Molecular Xpert MTB/RIF® test.** The cartridge contains a sample processing control (lyophilized *Bacillus globigii*) to ensure the proper processing of target bacteria and to monitor the presence of inhibitors, and five probes with a wild phenotype of *M. tuberculosis*. The sodium hydroxide- and isopropanol-containing reagent was added to the sample in a 2:1 (v/v) ratio. This mixture was stirred by vortex for 5 seconds and subsequently incubated at room temperature for 15 minutes until the sample was completely homogeneous. Then, 2 ml were transferred to the Xpert MTB/RIF® cartridge, which was then loaded into the GeneXpert® instrument where DNA extraction, real-time PCR and detection processes are fully automated. Each clinical sample was tested once regardless of the outcome.

The implementation protocol and results analysis were done using the GeneXpertDx System software version 4.3. The detection of the five specific probes for *M. tuberculosis* complex *rpoB* gene was considered a positive result. Trials in which the *M. tuberculosis* signal was negative but positive for the extraction and amplification internal control were considered as a negative result. Rifampin resistance was determined by failed or delayed hybridization of at least one or more *rpoB* gene specific probes.

**Conventional susceptibility testing.** The multiple proportions method is the test used to detect sensitivity of frontline anti-TB drugs (isoniazid, rifampin, ethambutol and streptomycin). Respiratory samples were sent to the Laboratorio de Salud

Pública de Antioquia, where the test has been standardized according to the process described by Canetti and Grosset (10).

### Statistical analysis

Microsoft Excel® 2010 was used for the database and to calculate the frequencies and the measures of central tendency. Epidat 3.1 software (Panamerican Health Organization) was used for calculations of validity: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio and kappa coefficient. Test sensitivity, specificity and predictive values were calculated with reference to the culture in solid medium.

### Results

From September, 2010, to December, 2011, 152 patients were screened, out of which 108 were eligible for the study and 103 were included in the analysis, as shown in figure 1. The bronchoalveolar lavage represented 61% (63) of the samples, sputum, 35% (36) and tracheal aspirate, 4% (4). Seropositivity for the human immunodeficiency

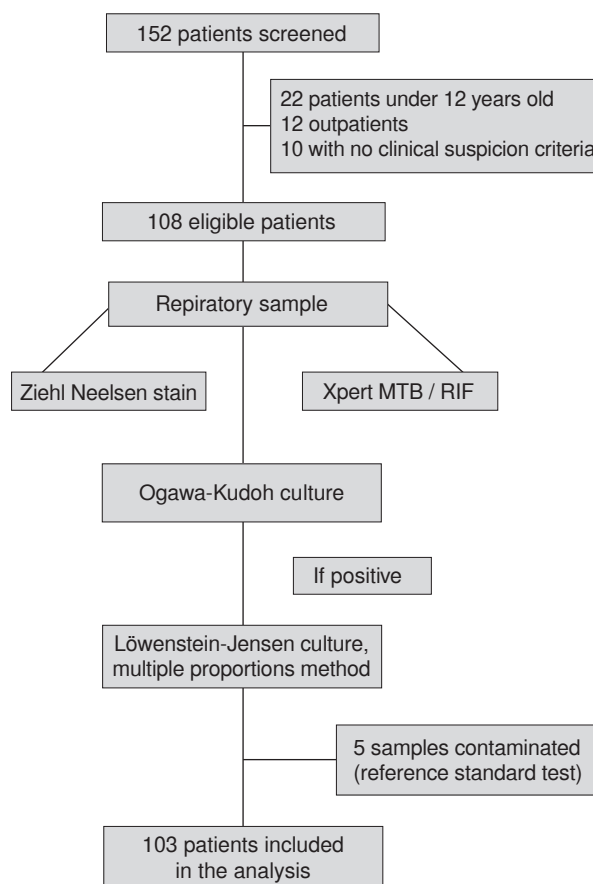


Figure 1. Study algorithm

virus (HIV) was found in 19.4% of the patients, with a median CD4 of 40x $\mu$ L; no history of tuberculosis or antiretroviral therapy was found (ART) (table 1).

The Xpert MTB/RIF® test was positive in 35 of the 103 samples. In 5.6% (6) of the cases the test was positive with negative culture while 2.9% (3) were culture-positive PCR-negative cases (table 2).

The overall sensitivity of the Xpert MTB/RIF® test was 91%, specificity, 92%, PPV, 83% and NPV, 96%. Its performance in samples with positive and negative ZN staining is shown in table 3. Of the 35 positive samples, 91.4% (31) were sensitive to RIF and 8.6% (3) were resistant. The results regarding sensitivity and resistance to rifampin were concordant with susceptibility testing using the multiple proportions method (Kappa = 1 p <0.0001).

## Discussion

The Xpert MTB/RIF® test is a useful tool for early diagnosis and treatment of pulmonary tuberculosis. Having the results regarding rifampin resistance in

two hours allows choosing the therapy appropriately, and thus reducing the probability of resistance to other drugs during treatment, as well as decreasing the time of patient infectivity and the appearance of aftermath. Its relevance as a diagnostic tool is foremost when respiratory samples are ZN-negative because it reduces the time to start treatment and it is not necessary to wait for culture results (10-12).

The Xpert MTB/RIF® test has high specificity to detect only *M. tuberculosis* DNA. It has been evaluated in 89 non-tuberculosis bacteria, fungi and viruses with no false positives (13). The last steps of the assay (polymerase chain reaction) are highly susceptible to samples contamination, but as these are kept in a sealed container, there is no possibility of contamination and, therefore, of having false positives.

The interpretation of test results in a clinical patient context is very important. The Xpert MTB/RIF® test cannot differentiate between live and dead *M. tuberculosis* bacilli (e.g., patients in treatment or previously treated). Given that there is no perfect gold standard, the decision whether to treat or not a patient when the test is positive and the culture is negative depends on the clinical pre-test probability. In a study of 105 patients with culture-negative samples that had been treated for tuberculosis on the basis of clinical symptoms, 29.3% had positive results on the Xpert MTB/RIF test (6).

There are some limitations in most hospitals "real" work scenarios in developing countries, as they do not meet the ideal conditions established by the most important test-validation studies.

Hospital Universitario San Vicente sees a large number of patients; there is, therefore, a significant amount of clinical samples to process in the laboratory, but limited availability of additional staff for reading the smears. These conditions may result in having to reduce the recommended time for reading

**Table 1.** General characteristics

	Total number of patients (N=103)
Median age (IQR)	37.5 (27-49)
Gender: Male/Female (%)	45 (43.6%) - 58 (56.4%)
HIV- / HIV+ (%)	83 (80.6%) / 20 (19.4%)
Respiratory samples: BAL/Sputum/TA	63/36/4
<b>HIV</b>	<b>20</b>
CD4 x $\mu$ L (IQR)	40 (20.5 -55)
ART Yes/No	0/20
History of tuberculosis	0
Caverns in Rx or CT scan	0
<b>No HIV:</b>	<b>83</b>
History of tuberculosis	8
Caverns in Rx or CT scan	10
Transplant	8

Rx: radiography, CT: computed tomography, BAL: bronchoalveolar lavage, TA: tracheal aspirate, IQR: interquartile range, ART: antiretroviral therapy, HIV: human immunodeficiency virus

**Table 2.** Interpretation of ZN, Xpert MTB/RIF® and cultures results in the study population

Number of patients (%)	Reference result (Ogawa-Kudoh)	Xpert MTB/RIF®	Ziehl Neelsen Respiratory samples	Interpretation
16 (15.5)	+	+	+	Full agreement
13 (12.6)	+	+	-	False negative Ziehl Neelsen
2 (1.94)	+	-	-	False negative Xpert/Ziehl Neelsen
64 (62.2)	-	-	-	Full agreement
0	-	+	+	False positive Xpert/Ziehl Neelsen
1 (0.97)	-	-	+	False positive Ziehl Neelsen
6 (5.82)	-	+	-	False positive Xpert
1 (0.97)	+	-	+	False negative Xpert



**Table 3.** Sensitivity, specificity, PPV and NPV of the Xpert MTB/RIF® test

N=103	General (CI)	Positive Ziehl Neelsen (CI)	Negative Ziehl Neelsen (CI)
Sensitivity %	91 (0.73-0.97)	94 (0.69-0.99)	87 (0.58-0.97)
Specificity %	92 (0.81-0.96)	100 (0.05-0.89)	91 (0.81-0.96)
PPV	83 (0.65-0.92)	100 (0.75-0.99)	68 (0.43-0.86)
NPV	96 (0.86-0.98)	50 (0.02-0.97)	97 (0.88-0.99)

PPV: Positive predictive value, NPV: Negative predictive value

each sputum sample (15 minutes) (14). In our case, we did not perform routine sputum centrifugation, which reduces the sensitivity of the method by 18% (15); additionally, we had no availability of fluorescence microscopy for processing samples with auramine/rhodamine staining. These conditions may have resulted in some samples classified as ZN-negative samples that could have been actually positive, the explanation being an increased sensitivity of the Xpert test in our ZN-negative samples compared with more controlled multicenter studies, since its performance is directly proportional to the bacterial load in the sample (6,7).

Another aspect to be considered is the culture media used. In most of the research studies published two culture media were used to assess the performance of the Xpert MTB/RIF® test: the Löwenstein-Jensen solid medium and the BACTEC MGIT automated liquid medium; however, many hospitals only have the Ogawa-Kudoh solid medium. It is known that the use of solid and liquid culture media can increase the sensitivity by 11% or more (16,17). The conventional culture medium requires 10<sup>2</sup> mycobacteria to detect positivity and the PCR requires only 10 or more mycobacteria. It may have happened that as only solid medium was used, Xpert MTB/RIF® test results in some samples were positive with negative culture and this could have been wrongly interpreted as false positives. There is little information in the literature on the clinical outcome of these patients over time (as regards developing the disease or not) (18). In this study we did not conduct clinical or microbiological monitoring over time. We used the Ogawa-Kudoh medium to evaluate the performance of the Xpert MTB/RIF® test which, to our knowledge, had not been done previously. The Ogawa-Kudoh medium has shown to have the same performance as the Löwenstein-Jensen medium for the diagnosis of *M. tuberculosis* infection (19).

Considering the above, in ZN-negative samples from our population the sensitivity was higher than that reported in studies conducted under “ideal”

conditions. Performance in ZN-positive samples is similar to that in previous reports (6,7). A sensitivity of 87% and a specificity of 91%, with an NPV of 97% in ZN-negative samples, makes the test a very useful tool to rule out the disease in the conditions described.

Larger studies (including pharmacoeconomic studies) are required to assess the true impact of the massive implementation of this test as a diagnostic method in our population, as it has the great advantage of requiring little preparation and handling of the samples; it allows the processing of multiple samples in time, and it is a fully automated method. Its cost (approximately USD\$250 per test in Colombia), however, is a disadvantage.

It should be noted that we included few HIV-positive cases and this may be a limitation to extrapolate the data to this group of patients. However, it seems that the diagnostic yield in this population may be better (20).

We found a high correlation between the gold standard method (proportion method) and molecular testing to detect sensitivity/resistance to RIF, similarly to that described by other studies, thus confirming that it is a reliable and reproducible test (6,7,16,20); this allows to detect early resistance and to initiate a prompt treatment preventing the spread or selection of resistant strains due to inadequate treatment. The national surveillance study on resistance to anti-TB drugs conducted in Colombia during 2004 and 2005 showed a prevalence of MDR-TB among untreated patients of 2.38% (95% CI 1.58 - 3.57) and of 50.6% among patients classified in Category I treatment failure (basic scheme for new tuberculosis patients) (21). In a recent study, a molecular test reduced MDR-TB detection time from 67.3 days (solid medium) and 21.6 days (liquid medium) to less than 5 days (4.2 days) compared to phenotypic methods (22).

In conclusion, the Xpert MTB/RIF® test is an additional tool for diagnosing pulmonary tuberculosis in developing countries which shows a better performance in ZN-negative samples than what was previously reported in the literature. As has been reported, the outcome of RIF-resistance or sensitivity detection through the identification of mutations in the *rpoB* gene is consistent with the phenotypic testing, which means the test is reliable and reproducible.

### Acknowledgements

To Hilda Álvarez, bacteriologist from the *Laboratorio de Salud Pública de Antioquia*, for performing the anti-TB drug susceptibility testing.

## Conflicts of interest

The authors declare that there were no conflicts of interest in conducting this study.

## Funding

This project was funded by the research unit of the Hospital Universitario San Vicente Fundación.

## References

1. **Organización Mundial de la Salud.** Control mundial de la tuberculosis - Informe OMS 2012. Fecha de consulta: 10 de febrero de 2013. Disponible en: [http://www.who.int/tb/publications/global\\_report/es/index.html](http://www.who.int/tb/publications/global_report/es/index.html).
2. **World Health Organization.** WHO monitoring of Xpert MTB/RIF roll-out. Fecha de consulta: 20 de septiembre de 2013. Disponible en: <http://www.who.int/tb/laboratory/mtbrifrollout/en/>.
3. **Raviglione MC1, Uplekar MW.** WHO's new Stop TB Strategy. *Lancet*. 2006;367:952-5. [http://dx.doi.org/10.1016/S0140-6736\(06\)68392-X](http://dx.doi.org/10.1016/S0140-6736(06)68392-X)
4. **English R, Bachmann MO, Bateman ED, Zwarenstein MF, Fairall LR, Bheekie A, et al.** Diagnostic accuracy of an integrated respiratory guidelines in identifying patients with respiratory symptoms requiring screening for pulmonary tuberculosis: A cross-sectional study. *BMC Pulm Med*. 2006;6:22. <http://dx.doi.org/10.1186/1471-2466-6-22>
5. **World Health Organization.** Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Fecha de consulta: 10 de febrero de 2013. Disponible en: [http://new.paho.org/hq/dmdocuments/2011/Hoja\\_Ruta\\_%20Introducci%3f3n\\_Xpert\\_MTB-RIF.pdf](http://new.paho.org/hq/dmdocuments/2011/Hoja_Ruta_%20Introducci%3f3n_Xpert_MTB-RIF.pdf).
6. **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;363:1005-15. <http://dx.doi.org/10.1056/NEJMoa0907847>
7. **Lawn S.** TB and HIV: How can we reduce mortality? *J Int AIDS Soc*. 2012;15(Suppl.4):18074. <http://dx.doi.org/10.7448/IAS.15.6.18074>
8. **Álvarez-Uria G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy R.** Rapid diagnosis of pulmonary and extrapulmonary tuberculosis in HIV-infected patients. Comparison of LED fluorescent microscopy and the GeneXpert MTB/RIF assay in a District Hospital in India. *Tuberc Res Treat*. 2012;2012:932862. <http://dx.doi.org/10.1155/2012/932862>
9. **del Corral H, París SC, Marín ND, Marín DM, López L, Henao HM, et al.** IFN gamma response to *Mycobacterium tuberculosis*, risk of infection and disease in household contacts of tuberculosis patients in Colombia. *PLoS One*. 2009;4:e8257. <http://dx.doi.org/10.1371/journal.pone.0008257>
10. **Canetti G, Rist N, Grosset J.** Measurement of sensitivity of the tuberculous bacillus to antibacillary drugs by the method of proportions. Methodology, resistance criteria, results and interpretation. *Rev Tuberc Pneumol (Paris)*. 1963;27:217-72.
11. **Falzon D, Jaramillo E, Schünemann HJ, Arentz M, Bauer M, Bayona J, et al.** WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. *Eur Respir J*. 2011;38:516-28. <http://dx.doi.org/10.1183/09031936.00073611>
12. **Fitzgerald D, Haas DW.** Principles and Practice of Infectious Diseases. *Mycobacterium tuberculosis*. 7th edition. Philadelphia: Churchill Livingstone; 2009. p. 3129-63.
13. **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;48:2495-501. <http://dx.doi.org/10.1128/JCM.00128-10>
14. **Whitelaw AC, Sturm WA.** Microbiological testing for *Mycobacterium tuberculosis*. In: Schaaf S, Zumla A, editors. *Tuberculosis: A comprehensive clinical reference*. London: Saunders Elsevier; 2009. p. 169-78.
15. **Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al.** Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: A systematic review. *Lancet Infect Dis*. 2006;6:664-74. [http://dx.doi.org/10.1016/S1473-3099\(06\)70602-8](http://dx.doi.org/10.1016/S1473-3099(06)70602-8)
16. **Chien HP, Yu MC, Wu MH, Lin TP, Luh KT.** Comparison of the BACTEC MGIT 960 with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. *Int J Tuberc Lung Dis*. 2000;4:866-70.
17. **Chew WK, Lasaitis RM, Schio FA, Gilbert GL.** Clinical evaluation of the Mycobacteria Growth Indicator Tube (MGIT) compared with radiometric (Bactec) and solid media for isolation of *Mycobacterium* species. *J Med Microbiol*. 1998;47:821-27. <http://dx.doi.org/10.1099/00222615-47-9-821>
18. **Kibiki GS, Mulder B, van der Ven AJ, Sam N, Boeree MJ, van der Zanden A, et al.** Laboratory diagnosis of pulmonary tuberculosis in TB and HIV endemic settings and the contribution of real time PCR for *M. tuberculosis* in bronchoalveolar lavage fluid. *Trop Med Int Health*. 2007;12:1210-7. <http://dx.doi.org/10.1111/j.1365-3156.2007.01907.x>
19. **Rivas C, Coitinho C, Dafond V, Corbo M, Baldjian M.** Performance of the Ogawa-Kudoh method for isolation of mycobacteria in a laboratory with large-scale workload. *Rev Argent Microbiol*. 2010;42:87-90. <http://dx.doi.org/10.1590/S0325-75412010000200003>
20. **Carriquiry G, Otero L, González-Lagos E, Zamudio C, Sánchez E, Nabeta P, et al.** A diagnostic accuracy study of Xpert®MTB/RIF in HIV-positive patients with high clinical suspicion of pulmonary tuberculosis in Lima, Perú. *PLoS One*. 2012;7:e44626. <http://dx.doi.org/10.1371/journal.pone.0044626>
21. **Garzón MC, Angée DY, Llerena C, Orjuela DL, Victoria JE.** Vigilancia de la resistencia del *Mycobacterium tuberculosis* a los fármacos antituberculosos, Colombia 2004-2005. *Biomédica*. 2008;28:319-26. <http://dx.doi.org/10.7705/biomedica.v28i3.71>
22. **Tukvadze N, Kempker RR, Kalandadze I, Kurbatova E, Leonard MK, Apsindzelashvili R, et al.** Use of a molecular diagnostic test in AFB smear positive tuberculosis suspects greatly reduces time to detection of multidrug resistant tuberculosis. *PLoS One*. 2012;7:e31563. <http://dx.doi.org/10.1371/journal.pone.0031563>