

**Dried Culture Spots for Xpert MTB/RIF  
External Quality Assessment: Results of a  
Phase 1 Pilot Study in South Africa**

L. E. Scott, N. Gous, B. E. Cunningham, B. D. Kana, O.  
Perovic, L. Erasmus, G. J. Coetzee, H. Koornhof and W.  
Stevens

*J. Clin. Microbiol.* 2011, 49(12):4356. DOI:  
10.1128/JCM.05167-11.

Published Ahead of Print 5 October 2011.

---

Updated information and services can be found at:  
<http://jcm.asm.org/content/49/12/4356>

---

*These include:*

**REFERENCES**

This article cites 19 articles, 8 of which can be accessed free at:  
<http://jcm.asm.org/content/49/12/4356#ref-list-1>

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new  
articles cite this article), [more»](#)

---

Information about commercial reprint orders: <http://jcm.asm.org/site/misc/reprints.xhtml>  
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

## Dried Culture Spots for Xpert MTB/RIF External Quality Assessment: Results of a Phase 1 Pilot Study in South Africa<sup>∇</sup>

L. E. Scott,<sup>1\*</sup> N. Gous,<sup>1</sup> B. E. Cunningham,<sup>1</sup> B. D. Kana,<sup>2</sup> O. Perovic,<sup>3</sup>  
L. Erasmus,<sup>4</sup> G. J. Coetzee,<sup>4</sup> H. Koornhof,<sup>4</sup> and W. Stevens<sup>1,5</sup>

Department of Molecular Medicine and Haematology, Faculty of Health Sciences, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa<sup>1</sup>; Centre of Excellence for Biomedical TB Research,<sup>2</sup> Microbiology External Quality Assessment Reference Unit at NICD and CMID, University of the Witwatersrand, Johannesburg, South Africa<sup>3</sup>; and National TB Reference Laboratory (NTBRL),<sup>4</sup> National Priority Program of the NHLS,<sup>5</sup> Johannesburg, South Africa

Received 19 July 2011/Returned for modification 24 August 2011/Accepted 25 September 2011

**Implementation of Xpert MTB/RIF requires quality assessment. A pilot program using dried culture spots (DCSs) of inactivated *Mycobacterium tuberculosis* is described. Of 274 DCS results received, 2.19% generated errors; the remainder yielded 100% correct *Mycobacterium tuberculosis* detection. The probe A cycle threshold ( $C_T$ ) variability of three DCS batches was  $\leq 3.47$ . The study of longer-term DCS stability is ongoing.**

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) (1, 3–5, 9, 12, 13, 15, 19, 25) for the diagnosis of *Mycobacterium tuberculosis* has recently been endorsed by the WHO (28), and recommendations for data collection to quantify the impact of this GeneXpert (GX) technology are provided (26). Guidance, however, with respect to appropriate external quality assessment (EQA) programs is lacking (17). Current international tuberculosis (TB) EQA programs focus on microscopy, culture, and susceptibility testing laboratories (24) and highlight the difficulties in expansion due to labor-intensive preparatory work and the high cost and regulations associated with shipping drug-resistant isolates (27).

Criteria for a verification (“fit for purpose”) and EQA program suited to the characteristics of the Xpert MTB/RIF assay (3, 8) will require the following elements. (i) The testing material must contain whole *M. tuberculosis* (8). (ii) Transportation of EQA material needs to be safe. (iii) The testing procedure needs to be safe and compatible with the Xpert MTB/RIF current testing protocol. (iv) Health care workers who do not have laboratory skills must be able to perform the testing in nonlaboratory settings. (v) Finally, the programs will need to be cost-effective and sustainable. Such a program using whole inactivated *M. tuberculosis* spotted onto filter paper was developed and piloted in South Africa as part of the National Health Laboratory Service (NHLS) GX rollout.

*M. tuberculosis* was obtained from (i) pooled samples from 20 microbial growth incubation tubes (MGIT) of rifampin (RIF)-susceptible clinical isolates and tested with the MTB-DRplus (Hain Life Sciences), (ii) 20 pooled MGIT cultures comprising American Type Culture Collection (ATCC) strain

S-MYCTU-02-P2 (ATCC 25177 [H37Ra]) and well-characterized local clinical strain MYCTU 15, and (iii) the ATCC 25618 (H37Rv) laboratory strain grown for single-cell-organism suspensions (11). The MGIT cultures S-MYCTU-02-P2 and MYCTU 15 and clinical isolates were pooled in their respective batches (with strains kept separate and not mixed), centrifuged ( $3,000 \times g$  for 15 min at 4°C) to pellet cells, and resuspended in 40 ml phosphate-buffered saline (PBS) followed by addition of 80 ml (2:1 ratio of buffer to culture) of the Xpert sample reagent (SR) buffer. For the H37Rv strain, 200 ml of culture was harvested (by centrifugation at  $3,500 \times g$ ) at room temperature for 10 min, and cells were resuspended in PBS to 40 ml followed by addition of 80 ml SR buffer (2:1 ratio of buffer to cells). Both MGIT-grown and H37Rv strain cultures were inactivated in SR buffer for 2 h at room temperature, with intermittent mixing. The inactivated material was washed twice with sterile PBS and resuspended in final volumes of 10 ml (S-MYCTU-02-P2 and MYCTU 15) and 40 ml (H37Rv) PBS. For confirmation of inactivation, washed cultures (0.5 ml) were reinoculated into new MGIT tubes in Bactec cabinets for 42 days. These inactivated bulk stocks were enumerated by flow cytometry (FC500 using Flow count microspheres; Beckman Coulter) and tested with the Xpert MTB/RIF assay. The cycle threshold ( $C_T$ ) values of the semi-quantitative categories (high,  $C_T$  of  $<16$ ; medium,  $C_T$  of 16 to 22; low,  $C_T$  of 22 to 28; and very low,  $C_T$  of  $>28$ ) were recorded for probe A and were compared to the flow cytometry enumeration score. Dilutions that generated a medium ( $C_T$  of 16 to 22) qualitative Xpert MTB/RIF result were used to prepare the dried culture spots (DCSs).

DCSs were prepared by spotting 25- $\mu$ l amounts of inactivated culture material onto Whatman 903 filter cards (Merck) together with 2  $\mu$ l of DNA loading dye (Sigma-Aldrich) per spot for visualization purposes, as illustrated in Fig. 1, and dried for 1 h at room temperature before being placed in sealed plastic bags with a desiccant sachet (Sigma-Aldrich).

\* Corresponding author. Mailing address: Department of Molecular Medicine and Haematology, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, South Africa. Phone: 27114898567. Fax: 27114845812. E-mail: lesley.scott@nhls.ac.za.

<sup>∇</sup> Published ahead of print on 5 October 2011.

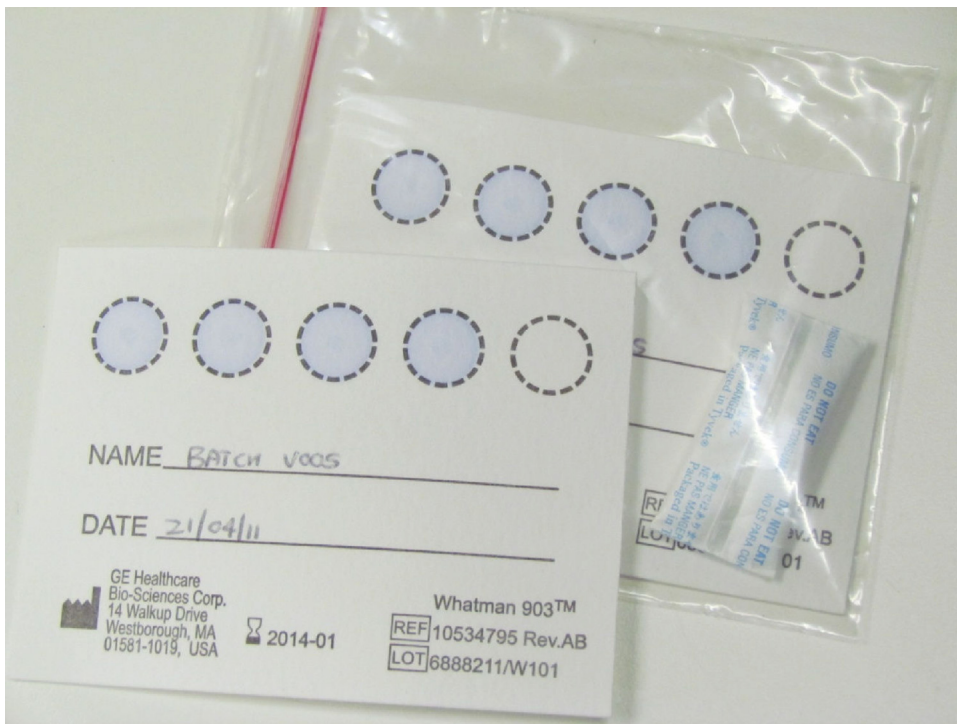


FIG. 1. A sample of the DCSs on filter cards and in plastic transport bags with desiccant sachets. Four DCSs on a card containing inactivated *M. tuberculosis* culture are visualized by the blue dye.

These were couriered ( $n = 16$ ), hand delivered ( $n = 10$ ), or surface mailed (repeat DCSs to 4 sites) to various participating sites, where each spot was cut (using a sterile pair of scissors) into a 50-ml standard laboratory Nunc centrifuge tube (AEC

Amersham), and 2.8 ml SR buffer (to ensure there was a sufficient 2-ml concentration to pipette into the Xpert MTB/RIF cartridge after the DCS incubation) was added to the tube. The tubes were vortexed (or hand shaken by swirling

TABLE 1. Performance of the three DCS batches on 286 GX modules

Parameter	Result for DCS batch no.:		
	V002	V004	V005
<i>M. tuberculosis</i> bulk culture material	MGIT clinical controls (RIF-sensitive <i>M. tuberculosis</i> )	MGIT ATCC strain (RIF-sensitive <i>M. tuberculosis</i> )	H37 laboratory strain (RIF-sensitive <i>M. tuberculosis</i> )
No. of GX modules tested by DCS	49 (all RIF-sensitive <i>M. tuberculosis</i> )	173 (all RIF-sensitive <i>M. tuberculosis</i> ) <sup>a</sup>	64 (all RIF-sensitive <i>M. tuberculosis</i> )
No. of errors <sup>b</sup>			
Error 5007		1	
Error 5011	1	3	1
No. of DCSs for statistical analysis	48	157	63
% of testing in qualitative category:			
Very low	0	5.1	6.25
Low	26.53	47.13	42.19
Medium	69.39	47.77	48.44
High	2.04	0	1.56
$C_T$ for probe A			
Mean	20.75	22.58	21.89
SD	2.20	2.76	3.47
CV (%)	10.6	12.22	15.86

<sup>a</sup> A total of 161 modules returned results.

<sup>b</sup> Error 5011 refers to signal loss detected in an amplification curve, and error 5007 refers to a probe check failure.

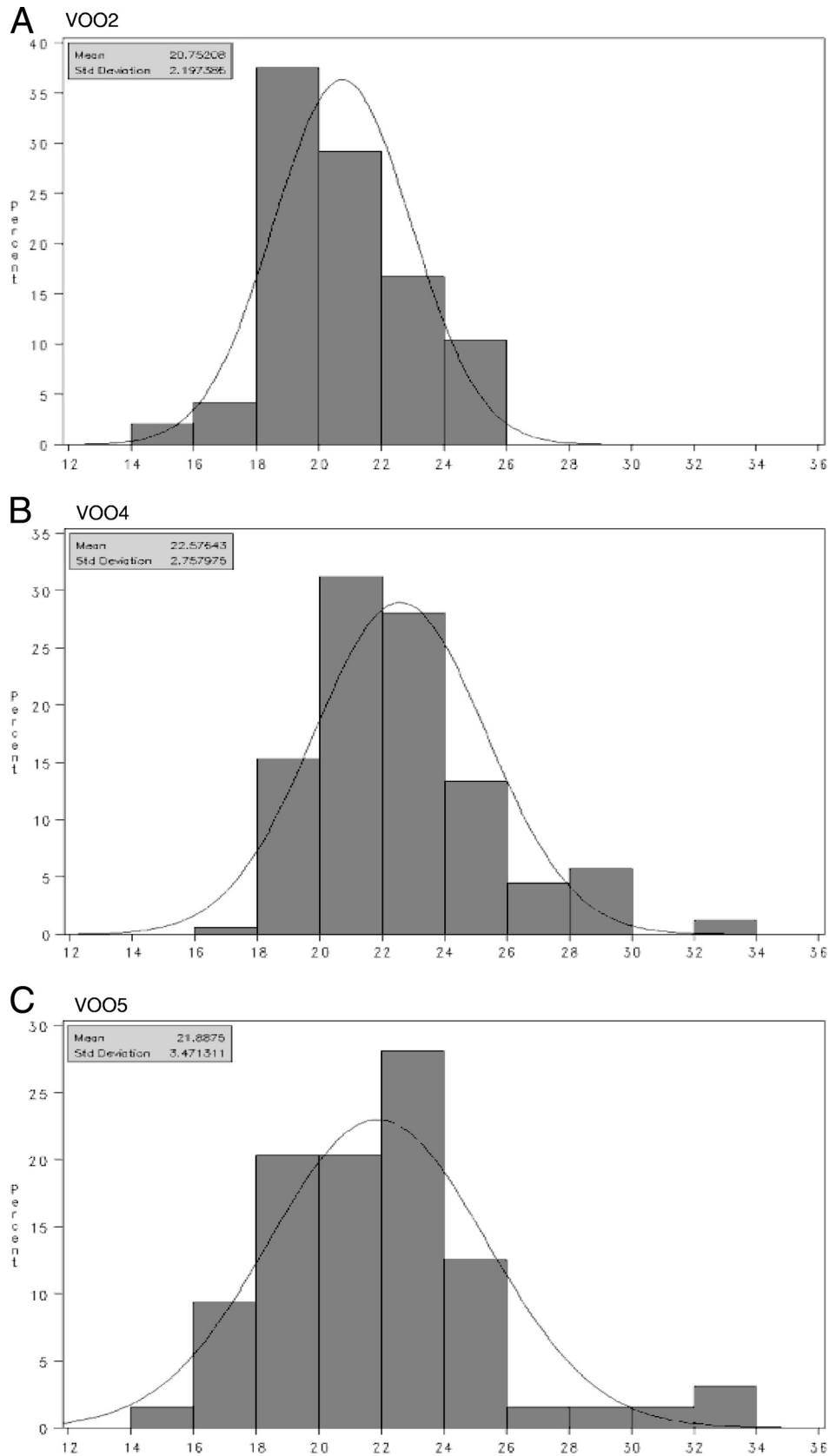


FIG. 2. Frequency distributions overlaid with normal curves of the  $C_T$  values for probe A from the three DCS batches. (A) Batch V002; (B) batch V004; (C) batch V005. The standard deviation and mean  $C_T$  values are represented in insets in each of the panels.

vigorously if no vortexer was available) and left at room temperature for 15 min with intermittent mixing. One DCS was then tested on each Xpert MTB/RIF module. The  $C_T$  mean, standard deviation, and coefficient of variation (CV) were calculated for probe A.

Three DCS batches were manufactured for 31 GXs: GX Infinity-48 ( $n = 1$ ), GX16 ( $n = 9$ ), and GX4 ( $n = 21$ ). A total of 286 DCSs were distributed to the 26 participating sites, and results were received for 274 DCSs, thereby identifying sites with nonconformities. Six testing errors (error no. 5011 [ $n = 5$ ] and 5007 [ $n = 1$ ]) were reported, and the remaining 268 DCSs generated results with 100% *M. tuberculosis* positivity and RIF sensitivity (Table 1). Probe A was the first probe to reach the amplification  $C_T$ , with similar standard deviations across three DCS batches with a  $C_T$  of  $\leq 3.47$ . Frequency distributions in Fig. 2 illustrate the greatest variability in batch V005 (CV of 15.86%) from the single-cell-generated culture.

National Xpert MTB/RIF implementation programs are challenged by determining the scope and composition of EQA panels and the infectious nature of *M. tuberculosis* material. This study provides a preliminary demonstration through the use of inactivated *M. tuberculosis* coupled with easier transportation of DCS material that an EQA program can be safely provided. The DCS material proved successful for verification of GX instruments and highlighted expected error code frequencies (2.1%) and site nonconformities.

Although this is a uniquely designed EQA program that appears so far suitable for Xpert MTB/RIF verification using different strains from different culture methods, the individual components are not unfamiliar to the field: filter paper has been used for the transportation and molecular testing of *M. tuberculosis* DNA (7, 14), and flow cytometry has been used for the analysis of *M. tuberculosis* (2, 10, 16, 18, 20–23). Flow cytometry has the advantage of rapidly and accurately identifying inactivated single whole bacterial cells, which circumvents conventional, time-consuming CFU enumeration methodologies. Enumeration of flow cytometric events can also be performed below the minimum McFarlane concentrations ( $1 \times 10^7$  CFU/ml) and could more accurately be used in strain mixing to test “dropout” or “delayed”  $C_T$ s (3). Flow cytometry is also available in settings that currently perform CD4 counting of HIV patients for treatment initiation and monitoring and therefore represent a platform and infrastructure already in place (6).

The variability in  $C_T$  values may result from the spotting technique, different DCS reconstitution techniques (including vortexing/hand shaking), and variability in the amount of SR buffer added to each DCS. Other sources of variability may be explained by *M. tuberculosis* clumping from the MGIT-grown cultures being better trapped by the Xpert MTB/RIF filter membrane, whereas an *M. tuberculosis* single cell ( $\sim 0.4 \mu\text{m}$  wide by  $1.0 \mu\text{m}$  long) may pass through the  $0.8\text{-}\mu\text{m}$  membrane pore. The advantage of single-cell-cultured material is that no sonication or declumping methods are required before flow cytometry enumeration and spotting.

Future design of an Xpert MTB/RIF EQA program could be similarly based on line probe assay programs using one pansusceptible strain, one RIF-monoresistant strain with a common *rpoB* mutation, one multidrug-resistant (MDR) strain, one nontuberculous mycobacterium (NTM) strain, and

a negative control (17), each placed on a DCS card and distributed 3 times per year.

This publication was made possible by the generous support of the American people through the U.S. Agency for International Development, the South Africa Tuberculosis and AIDS Training (SATBAT) program (National Institutes of Health/Fogarty International Center) (5U2RTW007370 and 5U2RTW007373), the National Research Foundation/Department of Science and Technology, and the South African Medical Research Council. We thank Beckman Coulter South Africa for the donation of the flow cytometry platform.

We thank Mara Gibson from Contract Laboratory Service.

The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the US government.

## REFERENCES

- Armand, S., P. Vanhuls, G. Delcroix, R. Courcol, and N. Lemaitre. 2011. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. *J. Clin. Microbiol.* **49**:1772–1776.
- Blackwood, K. S., et al. 2005. Viability testing of material derived from *Mycobacterium tuberculosis* prior to removal from a containment level-III laboratory as part of a Laboratory Risk Assessment Program. *BMC Infect. Dis.* **5**:4.
- Blakemore, R., et al. 2010. Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J. Clin. Microbiol.* **48**:2495–2501.
- Boehme, C. C., et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N. Engl. J. Med.* **363**:1005–1015.
- Boehme, C. C., et al. 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* **377**:1495–1505.
- Glencross, D. K., H. M. Aggett, W. S. Stevens, and F. Mandy. 2008. African regional external quality assessment for CD4 T-cell enumeration: development, outcomes, and performance of laboratories. *Cytometry B Clin. Cytom.* **74**(Suppl. 1):S69–S79.
- Guio, H., et al. 2006. Method for efficient storage and transportation of sputum specimens for molecular testing of tuberculosis. *Int. J. Tuberc. Lung Dis.* **10**:906–910.
- Helb, D., et al. 2009. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J. Clin. Microbiol.* **48**:229–237.
- Hillemann, D., S. Rusch-Gerdes, C. Boehme, and E. Richter. 2011. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *J. Clin. Microbiol.* **49**:1202–1205.
- Holm, C., T. Mathiasen, and L. Jespersen. 2004. A flow cytometric technique for quantification and differentiation of bacteria in bulk tank milk. *J. Appl. Microbiol.* **97**:935–941.
- Kana, B. D., et al. 2008. The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth in vitro. *Mol. Microbiol.* **67**:672–684.
- Malbruny, B., G. Le Marrec, K. Courageux, R. Leclercq, and V. Cattoir. Rapid and efficient detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory samples. *Int. J. Tuberc. Lung Dis.* **15**:553–555.
- Marlowe, E. M., et al. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. *J. Clin. Microbiol.* **49**:1621–1623.
- Miotto, P., F. Piana, G. B. Migliori, and D. M. Cirillo. 2008. Evaluation of the GenoCard as a tool for transport and storage of samples for tuberculosis molecular drug susceptibility testing. *New Microbiol.* **31**:147–150.
- Moure, R., et al. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. *J. Clin. Microbiol.* **49**:1137–1139.
- Norden, M. A., T. A. Kurzynski, S. E. Bownds, S. M. Callister, and R. F. Schell. 1995. Rapid susceptibility testing of *Mycobacterium tuberculosis* (H37Ra) by flow cytometry. *J. Clin. Microbiol.* **33**:1231–1237.
- Parsons, L. M., et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. *Clin. Microbiol. Rev.* **24**:314–350.
- Pina-Vaz, C., S. Costa-de-Oliveira, and A. G. Rodrigues. 2005. Safe susceptibility testing of *Mycobacterium tuberculosis* by flow cytometry with the fluorescent nucleic acid stain SYTO 16. *J. Med. Microbiol.* **54**:77–81.
- Rachow, A. 2010. Detection of *Mycobacterium tuberculosis* using the Cepheid Xpert MTB/RIF assay: a clinical validation study from Tanzania. 41st World Conference on Lung Health, Berlin, Germany.
- Reis, R. S., I. Neves, Jr., S. L. Lourenco, L. S. Fonseca, and M. C. Lourenco. 2004. Comparison of flow cytometric and Alamar Blue tests with the proportional method for testing susceptibility of *Mycobacterium tuberculosis* to rifampin and isoniazid. *J. Clin. Microbiol.* **42**:2247–2248.



21. **Sakamoto, C., N. Yamaguchi, and M. Nasu.** 2005. Rapid and simple quantification of bacterial cells by using a microfluidic device. *Appl. Environ. Microbiol.* **71**:1117–1121.
22. **Schellenberg, J., T. Blake Ball, M. Lane, M. Cheang, and F. Plummer.** 2008. Flow cytometric quantification of bacteria in vaginal swab samples self-collected by adolescents attending a gynecology clinic. *J. Microbiol. Methods* **73**:216–226.
23. **Soejima, T., K. Iida, T. Qin, H. Taniai, and S. Yoshida.** 2009. Discrimination of live, anti-tuberculosis agent-injured, and dead *Mycobacterium tuberculosis* using flow cytometry. *FEMS Microbiol. Lett.* **294**:74–81.
24. **Van Deun, A., et al.** 2009. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. *J. Clin. Microbiol.* **47**:3501–3506.
25. **Van Rie, A., L. Page-Shipp, L. Scott, I. Sanne, and W. Stevens.** 2010. Xpert MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? *Expert Rev. Mol. Diagn.* **10**:937–946.
26. **WHO.** March 2011. Rapid implementation of the Xpert MTB/RIF diagnostic test. Technical and operation 'how-to' practical considerations. WHO, StopTB. WHO, Geneva, Switzerland. <http://www.stoptb.org>.
27. **WHO.** 2004. Laboratory biosafety manual, 3rd ed. HO/CDS/CSR/LYO/2004.11. WHO Geneva, Switzerland. <http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf>.
28. **WHO.** 8 December 2010, posting date. WHO endorses new rapid tuberculosis test. WHO, Geneva, Switzerland. [http://www.who.int/mediacentre/news/releases/2010/tb\\_test\\_20101208/en/index.html](http://www.who.int/mediacentre/news/releases/2010/tb_test_20101208/en/index.html).