Valuing the use of GeneXpert test as an unconventional approach to diagnose pulmonary tuberculosis

Amany Omar, Abd-Elazim Abo Elfadl, Yousef Ahmed, Moustafa Hosny

Background A rapid, highly sensitive, and qualitative diagnostic test will significantly reduce the incidence of tuberculosis (TB). GeneXpert test is the test that is supposed to play this role with those specifications.

Objective Our goal was to assess the precision of the GeneXpert test in the diagnosis of pulmonary TB.

Patients and methods This cross-sectional analytic study was carried out at Assiut University Hospital in collaboration with Assiut Chest Hospital, Assiut, Egypt. A total of 67 patients of suspected pulmonary TB were included. For microbiological examination, two sputum samples were obtained from each patient within the same day. One sample was collected at Assiut University Hospital and subjected to smear microscopy by Ziehl–Neelsen staining and culture on Lowenstein–Jensen media. The other sample was taken at Assiut Chest Hospital to be processed for GeneXpert *Mycobacterium tuberculosis*/rifampicin assay. The culture was applied as a confirmatory test to evaluate the Xpert *Mycobacterium tuberculosis*/rifampicin assay test.

Results GeneXpert had 95.9% sensitivity and 94.4% specificity in diagnosing pulmonary TB with the area under the curve of 0.95 and overall diagnostic accuracy of 95.5%. Ziehl–Neelsen examination had 65.3% sensitivity and 100% specificity with the area under the curve of 0.83 and overall diagnostic accuracy of 74.6. For smear-negative, culture-positive cases, GeneXpert showed sensitivity of 94.1%. False-positive GeneXpert for TB was recorded in just one patient.

Conclusion The GeneXpert test is accurate in diagnosing pulmonary TB and its greatest benefit is clearly demonstrated in smear-negative TB cases. However, the test is not free from some fallacies, even if they are a few, which draws our attention to the importance of the conventional culture for TB and the clinical correlation.

Egypt J Bronchol 2019 13:403–407

Egyptian Journal of Bronchology 2019 13:403–407

Keywords: Assiut, Egypt, GeneXpert, pulmonary tuberculosis, Ziehl–Neelsen

Department of Chest, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence to Yousef Ahmed, MD, Department of Chest, Faculty of Medicine, Assiut University Hospital, Assiut, Egypt. Tel: +20 102 503 3083; e-mail: yousef_ahmed1972@yahoo.com

Received 16 November 2018 **Accepted** 3 February 2019

Introduction

Tuberculosis (TB) remains one of the major health risks around the world that is associated with high morbidity and deaths [1,2]. The fast and precise determination of TB infection and disease is basic for a convenient start of treatment and, eventually, control of the malady. In clinical practice, however, TB can be difficult to diagnose, and early TB detection remains a challenge for physicians [3]. The diagnostic challenges can be further magnified in special situations such as extrapulmonary TB, childhood TB, and patients coinfected with HIV and TB [4]. A rapid, highly sensitive, and qualitative diagnostic test will significantly reduce the incidence of TB. GeneXpert test is the test that is supposed to play this role with those specifications. The GeneXpert assay is an automated molecular diagnostic test for TB. It can at the same time identify *Mycobacterium tuberculosis* (MTB) complex DNA and transformations related to rifampicin (RIF) resistance [5,6]. In this study, our goal was to assess the precision of the GeneXpert test in the diagnosis of pulmonary TB.

Patients and methods

This cross-sectional analytic study was carried out at Assiut University Hospital in collaboration with Assiut Chest Hospital, Assiut, Egypt. The patients were selected from those who sought medical advice at the Chest Outpatient Clinic of Assiut University Hospital from October 2016 to January 2018 after having agreed to participate. Our survey was approved by the Ethics Committee, Faculty of Medicine, Assiut University. Patients were suspected to have pulmonary TB based on the clinical data (e.g. prolonged cough, hemoptysis, night fever, night sweating, anorexia, and weight loss), the relative laboratory tests result (e.g. blood picture and erythrocyte sedimentation rate), and the radiological findings. Excluded from this study were patients suspected to have extrapulmonary TB, patients who proved to be tuberculous and started antituberculous treatment as well as those for whom we were unable to obtain appropriate spontaneous samples to be examined. All studied patients were subjected to thorough medical history, full clinical examination, and chest radiography. In addition some laboratory tests were performed such as complete blood picture and erythrocyte sedimentation rate. For microbiological examination, two sputum samples were obtained from

 DOI: 10.4103/ejb.ejb_88_18

each patient within the same day. One sample was collected at Assiut University Hospital and subjected to smear microscopy by Ziehl–Neelsen (ZN) staining and culture on Lowenstein–Jensen media following the known and agreed protocol [7,8]. The other sample was taken at Assiut Chest Hospital to be processed for GeneXpert MTB/RIF assay (Cepheid, Cepheid Inc., Sunnyvale, California, USA) following the manufacturer's instructions [9]. The culture was applied as a confirmatory test to evaluate the Xpert MTB/RIF assay test.

Statistical analysis

Statistical package for the social sciences (version 20; IBM, Armonk, New York, USA) was used for analyzing the collected data. Different statistical measures were calculated from the data collected for the GeneXpert test and smear microscopy to diagnose pulmonary TB. It should be noted that we did not plan to include in our statistical analysis any results from repeating any test if the first result was negative.

Results

A total of 67 patients of suspected pulmonary TB were included in this work. Bacteriological examination of those patients revealed that 49 (73.1%) of them had positive culture for TB and 18 (26.9%) had negative. The characterized data of the included patients are detailed in Table 1. Statistically, there were no significant differences between patients with positive and negative culture results, except for some symptoms such as night fever and night sweats, as well as some laboratory tests such as erythrocyte sedimentation rate. Regarding, microbiological examination of the sputum samples obtained from the studied patients, we found that in all patients with negative culture, ZN examination result was negative, but in those with positive cultures, ZN examination result was positive in 32 (65.3%) and negative in 17 (34.7%). Among 32 ZN smear-positive, GeneXpert-positive cases were 31 (96.9%). Of 17 ZN smear-negative patients 16 (94.1%) were found to be positive for acid-fast bacilli (AFB) by GeneXpert. Totally, GeneXpert was positive in 47 (95.9%) of those with positive culture result. False-positive GeneXpert for TB was recorded in just one patient who had a history of previously treated pulmonary TB (Tables 2 and 3). In addition, GeneXpert test revealed that five patients have RIF resistance. Table 4 and Figure 1 show the diagnostic accuracy of the GeneXpert assay in comparison with ZN examination in the studied patients. Based on the result of the culture, GeneXpert had 95.9% sensitivity

Table 1 Characteristics of patients enrolled in the study (*N*=67)

Variables	Positive culture for TB (*N*=49)	Negative culture for TB (*N*=18)
Age (years)	43.9±17.6	50.8±19.1
Sex		
Male	27 (55.1)	10 (55.5)
Female	22 (44.9)	8 (44.5)
Residence		
Rural	29 (59.2)	15 (83.3)
Urban	20 (40.8)	3 (16.7)
Occupation		
Farmer	17 (34.7)	4 (22.2)
Housewife	14 (28.6)	5 (27.8)
Office worker	4 (8.5)	5 (27.8)
Health care worker	5 (10.5)	0 (0)
Unemployed	9 (18.5)	4 (22.2)
Special habits		
Nonsmoker	20 (40.8)	5 (27.8)
Smoker	29 (59.2)	13(72.2)
Cigarette smoker	8 (16.3)	5 (27.8)
Goza smoker	16 (32.6)	7 (38.9)
Mixed (cigarette and Goza)	5 (10.2)	1 (5.6)
Addicts to cannabis and/or opium	4 (8.2)	0 (0)
Level of education		
Uneducated	7 (14.2)	4 (22.2)
Primary	19 (38.8)	5 (27.8)
Secondary	15 (30.7)	3 (16.7)
Tertiary	8 (16.3)	6 (33.3)
Symptoms		
Cough	49 (100)	18 (100)
Hemoptysis	19 (38.8)	5 (27.8)
Night fever	48 (98)	12 (66.7) *P*=0.02
Night sweating	47 (95.9)	12 (66.7) *P*=0.03
Anorexia	49 (100)	17 (94.4)
Weight loss	48 (98)	17 (94.4)
Dyspnea	9 (18.4)	4 (22.2)
Chest pain	7 (14.3)	0
History of chronic illness	27 (55.1)	6 (33.3)
Past history of TB	4 (8.1)	3 (16.6)
Family history of TB	5 (10.2)	1 (5.6)
Chest radiography findings		
Cavitary lesion	18 (36.8)	2 (11.1)
Noncavitary lesion	25 (51)	13 (72.2)
Both types of lesions	6 (12.2)	3 (16.7)
Nonpulmonary lesion	12 (24.4)	7 (38.9)
Unilateral lesion	34 (69.3)	11 (61.1)
Bilateral lesion	15 (30.7)	7 (38.9)
Complete blood picture		
Hemoglobin (mg/dl)	11.77±1.53	11±1.41
TLC ($\times 10^9$/l)	8.67±2.83	8.33±3.34
Platelets ($\times 10^9$/l)	285.34±121.18	286.94±119.40
ESR (ml/h)		
1st h	69.69±25.91	23±13.38 *P*=0.03
2nd h	97.2±24.38	42±24.14 *P*=0.01

Data are presented as *n* (%) and mean±SD. ESR, erythrocyte sedimentation rate; TB, tuberculosis; TLC, total leukocyte count.

Table 2 GeneXpert test and Ziehl–Neelsen examination results among patients with positive culture for tuberculosis

	Positive culture for AFB (*N*=49 patients)		
	Positive GeneXpert	Negative GeneXpert	Total
Positive culture for AFB (*N*=49 patients)			
Positive ZN smear	31	1	32/49 (65.3)
Negative ZN smear	16	1	17/49 (34.7)
Total	47/49 (95.9)	2/49 (4.1)	49

Data are presented as *n*/*N* (%). AFB, acid-fast bacilli; ZN, Ziehl–Neelsen.

Table 3 GeneXpert test and Ziehl–Neelsen examination results among patients with negative culture for tuberculosis

	Negative culture for AFB (*N*=18 patients)		Total
	Positive GeneXpert	Negative GeneXpert	
Negative culture for AFB (*N*=18 patients)			
Positive ZN smear	0	0	0
Negative ZN smear	1	17	18/18 (100)
Total	1/18 (5.6)	17/18 (94.4)	18

Data are presented as *n*/*N* (%). AFB, acid-fast bacilli; ZN, Ziehl–Neelsen.

Table 4 Accuracy of GeneXpert test and Ziehl–Neelsen examination in diagnosing pulmonary tuberculosis

Indices	GeneXpert test	ZN examination
Sensitivity (%)	95.9	65.3
Specificity (%)	94.4	100
Positive predictive value (%)	97.9	100
Negative predictive value (%)	89.5	51.4
Area under the curve	0.95	0.83
P value	<0.001	<0.001
Overall accuracy (%)	95.5	74.6

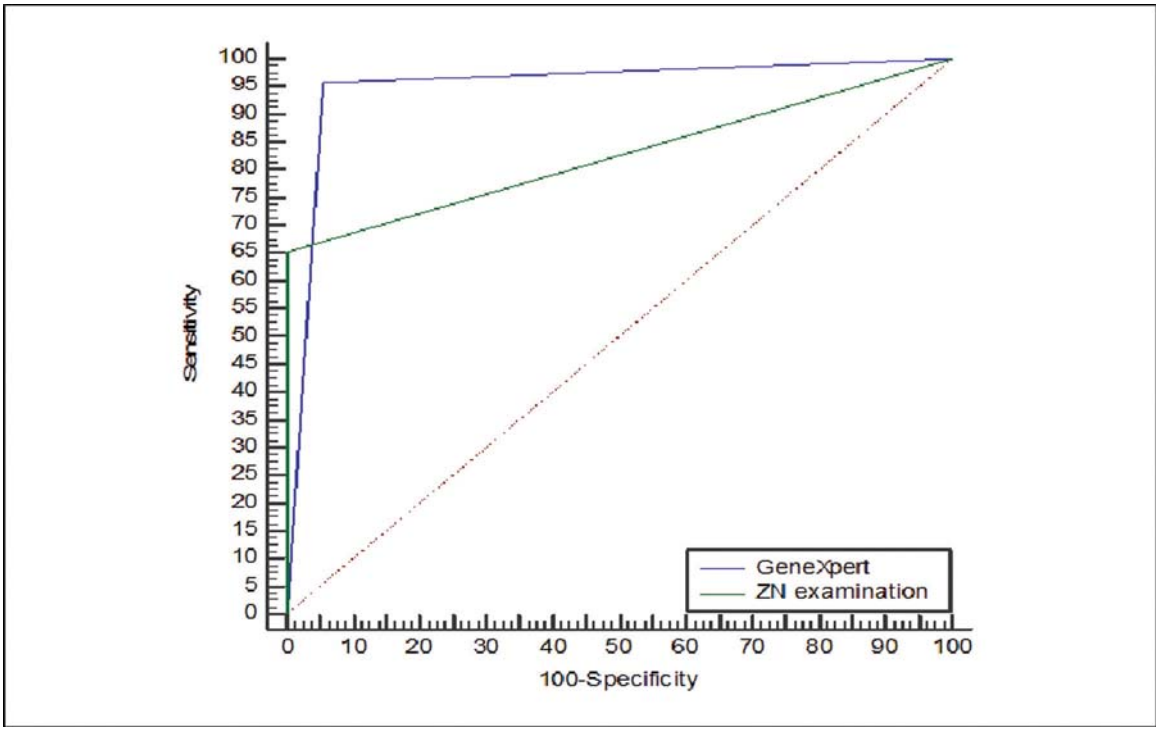
ZN, Ziehl–Neelsen.

and 94.4% specificity in diagnosing pulmonary TB with the area under the curve of 0.95 and overall diagnostic accuracy of 95.5%. On the contrary, ZN examination had 65.3% sensitivity and 100% specificity in diagnosing pulmonary TB with the area under the curve of 0.83 and overall diagnostic accuracy of 74.6.

Discussion

The new molecular technique Xpert MTB/RIF assay was approved by WHO in December 2010 for the

Figure 1



Accuracy of GeneXpert test and ZN examination in diagnosing pulmonary TB. TB, tuberculosis; ZN, Ziehl–Neelsen.

rapid detection of TB and RIF resistance. Various research studies have been carried out to examine GeneXpert's utility in different countries and populations. However, to our knowledge, no previous study has been conducted to evaluate the use of this technique on patients with TB at Assiut University Hospital, possibly owing to inaccessibility of the device required for this examination. Accordingly, we carried out this work in cooperation with Assiut Chest Hospital, Egyptian Ministry of Health where the necessary equipment is available. In this study, our goal was to assess the accuracy of the GeneXpert test and compare it with the sputum smear microscopy in the diagnosis of pulmonary TB using sputum culture for TB as a gold standard. Direct examination of sputum for AFB by ZN stained smears using conventional microscopy is a standard procedure for the diagnosis of pulmonary TB in countries with a high TB burden [10,11]. Although this method is very specific and is the fastest and most widely used method for detection of AFB in sputum, it has a low sensitivity compared with culture [12–16]. Its sensitivity is affected by many factors, such as disease prevalence and severity, quality of sample collection, the number of mycobacterium bacilli present in the specimen, and the quality of the examination (microscope operator expertise, time spent in smear examination, etc.) [17]. In a systematic review of AFB smear reports from around the world, reported sensitivity figures ranged from 32% to 94%, whereas the reported specificity ranged from 94 to 100% [18]. In our study, direct smear microscopy using ZN staining for AFB detection showed sensitivity of 65.3% and excellent specificity of 100%, which means that our results occurred within the observed ranges in most studies. GeneXpert MTB/RIF assay is a computerized test that can identify both TB and RIF resistance within 2 h, with minimal hands-on technical time. However, there was a contrast in sensitivity of the Xpert assay for identification of TB among different researches that can be explained by differences in participation criteria and procedures used to get sputum samples. A review of the diagnostic accuracy of Xpert TB found that when used as a preliminary test to replace the microscopy, its pooled sensitivity was 89% and specificity was 99% but when Xpert TB was used as an add-on for cases of negative smear microscopy, the sensitivity was only 67% and specificity was 99%. For smear-positive, culture-positive TB, Xpert MTB/RIF pooled sensitivity was 98%. For people with HIV infection, it had pooled sensitivity of 79% and for individuals without HIV infection, pooled sensitivity was 86% [19]. In late 2013, WHO prescribed using of GeneXpert assay test for the diagnosis of TB in some particular cases such as children and patients with certain forms of extrapulmonary TB [20]. A systematic review by Detjen *et al.* [21] revealed that Xpert offers sensitivity of 62% and specificity of 98% for the diagnosis of pulmonary TB in children. In terms of the Egyptian studies in this field, the reported sensitivity of Xpert test was 100% in cases of smear-positive, culture-positive TB and 66.6% in smear-negative, culture-positive cases, whereas its specificity for each category was 100% [22]. Moreover, Meawed and Shaker [23] reported 98.15% sensitivity for this technology. Moussa *et al.* [24] reported sensitivity of 93% and specificity of 98.3%. In our study, the overall sensitivity of the GeneXpert was 95.9% whereas the specificity was 94.4%. For smear negative, culture-positive cases, GeneXpert showed sensitivity of 94.1% whereas the reported sensitivity in smear-positive, culture-positive TB cases was 96.9%. Among the noticeable results of our study, one sputum sample was positive with GeneXpert but negative with Lowenstein–Jensen culture. The patient with this sample was initially treated with anti-TB drugs, which was later discontinued when the patient did not improve, and the culture was proven negative. This false-positive result was observed in a patient among those who gave a past history of TB. Several case reports have shown false-positive Xpert results in TB retreatment cases. Patients with former TB may have remaining DNA in sputum that can be extracellular or associated with nonintact cells, hence is not culturable. This can be confusing for nucleic acid amplification tests such as Xpert MTB/RIF and can lead to a possible false-positive result, which may trigger baseless treatment, delay reaching correct diagnosis, and increase health care costs [25–28]. Finally, although the main objective of our study was to assess the accuracy of the GeneXpert test in the detection of the AFB, we should not overlook the discovery of five cases of resistance to RIF by this test, but unfortunately we cannot ascertain the accuracy of the test in capturing such cases because there was no possibility of drug susceptibility test where our study was conducted.

Conclusion

The GeneXpert test is accurate in diagnosing pulmonary TB, and its greatest benefit is clearly demonstrated in smear-negative TB cases. However, the test is not free from some fallacies, even though few, which draws our attention to the importance of the conventional culture for TB and the clinical correlation. Moreover, this test cannot distinguish

between viable and nonviable microorganisms during the detection of MTB DNA, and therefore should not be used to monitor patients or efficacy of the treatment.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Maartens G, Wilkinson RJ. Tuberculosis. *Lancet* 2007; **370**:2030–2043.

2 Raviglione M, Sulis G. Tuberculosis 2015: burden, challenges and strategy for control and elimination. *Infect Dis Rep* 2016; **8**:33–37.

3 Alaarag AH, Mohammad OI, Farag NM. Diagnostic utility of serum adenosine deaminase level in the diagnosis of pulmonary tuberculosis. *Egypt J Bronchol* 2016; **10**:133–139.

4 World Health Organization. *Global tuberculosis report*. Geneva: WHO; 2016.

5 Munir MK, Anwar N, Iqbal R, Shabbir I, Nosheen S. Diagnosis of tuberculosis: molecular versus conventional method. *Pak J Med Res* 2011; **50**:50.

6 Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, *et al.* Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol* 2010; **48**:229–237.

7 International Union against Tuberculosis and Lung Disease. *Sputum examination for tuberculosis by direct microscopy in low income countries. Technical GUIDE*. 5th ed. Paris, France: IUATLD; 2000.

8 Chihota VN, Grant AD, Fielding K, Ndibongo B, van Zyl A, Muirhead D, *et al.* Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *Int J Tuberc Lung Dis* 2010; **14**:1024–1031.

9 Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, *et al.* Rapid molecular detection of tuberculosis and rifampin resistance. *N Eng J Med* 2010; **363**:1005–1015.

10 Tuberculosis Division International Union against Tuberculosis and Lung Disease. Tuberculosis bacteriology—priorities and indications in high prevalence countries: position of the technical staff of the Tuberculosis Division of the International Union against Tuberculosis and Lung Disease. *Int J Tuberc Lung Dis* 2005; **9**:355–361.

11 World Health Organization. *WHO laboratory services in tuberculosis control. Part II: microscopy*. Geneva: WHO; 1998. 30–32

12 Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Kallenius G, Lindquist L. Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV. *Trans R Soc Trop Med Hyg* 2000; **94**:677–680.

13 Murray SJ, Barrett A, Magee JG, Freeman R. Optimization of acid fast smears for the direct detection of mycobacteria in clinical samples. *J Clin Pathol* 2003; **56**:613–615.

14 Peterson EM, Nakasone A, Platon-DeLeon JM, Jang Y, de La Maza LM, Desmond E. Comparison of direct and concentrated acid-fast smears to identify specimens culture positive for *Mycobacterium spp*. *J Clin Microbiol* 1999; **37**:3564–3568.

15 Woods GL, Pentony E, Boxley MJ, Gatson AM. Concentration of sputum by cytocentrifugation for preparation of smears for detection of acid-fast bacilli does not increase sensitivity of the fluorochrome stain. *J Clin Microbiol* 1995; **33**:1915–1916.

16 Saceanu CA, Pfeiffer NC, McLean T. Evaluation of sputum smears concentrated by cytocentrifugation for detection of acid-fast bacilli. *J Clin Microbiol* 1993; **31**:2371–2374.

17 Somoskovi A, Hotaling JE, Fitzgerald M, O'Donnell D, Parsons LM, Salfinger M. Lessons from a proficiency testing event for acid-fast microscopy. *Chest* 2001; **120**:250–257.

18 Steingart KR, Henry M, Vivienne Ng, Hopewell PC, Ramsay A, Cunningham J, *et al.* Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis* 2006; **6**:570–581.

19 Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert (R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2014; **1**:CD009593.

20 Zumla A, George A, Sharma V, Herbert RHN, Ilton BM, Oxley A, *et al.* The WHO 2014 global tuberculosis report—further to go. *Lancet Glob Health* 2015; **3**:e10–e12.

21 Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, *et al.* Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. *Lancet Resp Med* 2015; **3**:451–461.

22 Darwish M, Magda AEW, Hazem A. Diagnostic assessment of Xpert MTB/RIF in sample of *Mycobacterium tuberculosis*. Egyptian patients. *Afr J Microbiol Res* 2013; **7**:5107–5113.

23 Meawed TE, Shaker A. Assessment of diagnostic accuracy of GeneXpert MTB/RIF in diagnosis of suspected retreatment pulmonary tuberculosis patients. *Egypt J Chest Dis Tub* 2016; **65**:637–641.

24 Moussa SM, Bayoumi FS, Ali AM. Evaluation of GeneXpert MTB/RIF assay for direct diagnosis of pulmonary tuberculosis. *Saudi Med J* 2016; **37**:1076–1081.

25 Theron G, Venter R, Calligaro G, Smith L, Limberis J, Meldau R, *et al.* Xpert MTB/RIF results in patients with previous tuberculosis: can we distinguish true from false positive results? *Clin Infect Dis* 2016; **62**:995–1001.

26 Boyles T, Hughes J, Cox V, Burton R, Meintjes G, Mendelson M. False-positive Xpert® MTB/RIF assays in previously treated patients: need for caution in interpreting results. *Int J Tuberc Lung Dis* 2014; **18**:876–878.

27 Kelly JD, Grace Lin SY, Barry PM, Keh C, Higashi J, Metcalfe JZ. Xpert MTB/RIF false detection of rifampin-resistant tuberculosis from prior infection. *Am J Respir Crit Care Med* 2014; **190**:1316–1318.

28 Stevenson DR, Biswas JR, Lievesley A, Quinn G, Buckley J, John L, *et al.* False-positive Xpert® MTB/RIF more than seven years after cure. *Int J Tuberc Lung Dis* 2015; **19**:1264–1265.