



FluoroType MTB system for the detection of pulmonary tuberculosis

To the Editor:

Diagnosis continues to be a major barrier for the control of tuberculosis (TB), especially in low- and middle-income countries (LMIC) [1]. The number of platforms for the molecular diagnosis of TB have increased in recent years and they can provide test results more rapidly than culture. Molecular assays are increasingly being used as alternative or adjunct methods to culture and smear microscopy, and modern systems seek to partially or fully automate the DNA extraction and amplification steps, increasing their suitability for resource-limited laboratories. One of these platforms, the GeneXpert MTB/RIF (Cepheid, USA), has a sensitivity of roughly 85% compared to culture [2] and has seen significant uptake in developing countries [3]. However, as a fully closed system, the DNA extracted during the process cannot be used for further downstream drug susceptibility testing (DST), which is crucial for patients with suspected drug-resistant TB.

FluoroType MTB (Hain Lifescience, Germany) is a new molecular test for the detection of TB that involves DNA amplification and detection *via* PCR in a closed system, with automated analysis, which can process up to 96 samples within 3 h. The extracted DNA can be used in genotypic DST line probe assays. DNA extraction can be performed manually or using an automated DNA extraction system (GenoXtract). A prospective assessment of the system in a low-incidence setting found the sensitivity and specificity to be 88.1% and 98.9%, respectively, in comparison with culture [4]. However, the system has not been evaluated in resource-poor settings.

We sought to evaluate the FluoroType MTB assay in Zankli Research Laboratory, Zankli Medical Centre, Abuja, Nigeria. Sputum samples were acquired from consecutive adult patients with a cough of ≥ 2 weeks duration attending TB diagnostic clinics at district hospitals within Abuja Federal Capital. Patients receiving TB treatment were excluded. Patients were asked to provide two sputum samples on the spot, one hour apart from one another. The minimum sputum volume accepted was 2 mL, and patients were asked to submit further specimens if this total was not reached. Samples were decontaminated using Petroff's method (4% NaOH). Specimens were examined using light-emitting diode fluorescence smear microscopy (LED-FM). One sputum specimen was split in half to test with FluoroType MTB and Xpert MTB/RIF and the second sample was cultured onto two tubes of solid Löwenstein-Jensen medium. DNA extraction for FluoroType was carried out using FluoroLyse; the manual extraction protocol developed by Hain, GenoXtract, was not available in the country. All tests were carried out by separate technicians, who were blinded to other results. All patients were offered HIV testing following the local guidelines for HIV counselling and testing. TB culture facilities in Zankli Medical Centre are quality controlled externally on a yearly basis by the Supranational TB laboratory in Milan.

The diagnostic accuracy of the FluoroType MTB assay was calculated using solid culture as the reference standard and described by LED-FM smear positivity, HIV status and Xpert MTB/RIF. Solid culture was chosen as the reference standard, despite its inferior sensitivity compared with liquid culture, in part to avoid contamination issues associated with liquid culture in this setting and also due to the difficulty in locally sourcing the required culture bottles. The agreement between FluoroType MTB and Xpert MTB/RIF was described using Kappa statistics.

A total of 296 patients had valid positive or negative culture results, with 70 (23.6%) and 226 (76.4%) being culture-positive and -negative, respectively. FluoroType MTB detected 62 culture-positive patients



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FluoroType MTB is a sensitive test for TB but specificity is low compared with fully integrated molecular systems <http://ow.ly/WhEO30b1luY>

Cite this article as: Obasanya J, Lawson L, Edwards T, *et al.* FluoroType MTB system for the detection of pulmonary tuberculosis. *ERJ Open Res* 2017; 3: 00113-2016 [<https://doi.org/10.1183/23120541.00113-2016>].

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(sensitivity 88.6%, 95% CI 78.7–94.9%), while Xpert MTB/RIF detected 55 cases (sensitivity 78.6%, 95% CI 67.13–87.5%) cases (table 1). The sensitivity of FluoroType MTB varied by smear status and was 100% (95% CI 92.9–100%) among smear-positive and 60% (95% CI 36.1–80.9%) among smear-negative cases, respectively. Similarly, sensitivity varied with HIV infection and was lower among HIV-positive (84.0%, 95% CI 63.9–95.5%) and higher among HIV-negative (100%, 95% CI 89.4–100%) patients.

FluoroType MTB was negative in 135 out of 226 culture-negative patients and positive in 91 (specificity 59.7%, 95% CI 53–66.2%) (table 1), while Xpert MTB/RIF was negative in 212 (93.8%, 95% CI 89.8–96.6%). FluoroType MTB specificity was significantly lower than previously reported, with two published studies reporting specificities of 96.4% [5] and 98.9% [4]. Specificity also varied by smear, HIV and Xpert MTB/RIF status. Specificity was lower among smear-positive culture-negative than in smear-negative culture-negative patients and among HIV-positive than HIV-negative patients, respectively ($p < 0.01$ for both), as shown in table 1. Positive FluoroType MTB results among culture-negative patients may indicate that FluoroType is more sensitive than culture. However, as most of these patients were also Xpert MTB/RIF negative, it is more likely that most of them were false-positive. Former evaluations were carried out in European settings, where laboratory facilities and experience in molecular techniques are more established. The low specificity in our setting suggests that resource-poor environments can present difficulties for the implementation of fairly complex molecular tests [6]. Of the 91 culture-negative samples that were positive by FluoroType MTB, 74 were negative by microscopy and Xpert MTB/RIF, and most laboratories would consider these patients likely to be true-negative. A further seven culture-negative samples were positive by both microscopy and Xpert MTB/RIF and positive by FluoroType MTB, suggesting that culture also missed some patients. The overall agreement between the FluoroType MTB and Xpert MTB/RIF was 69.6% (Kappa 0.355), with 84.3% agreement in culture-positive (Kappa 0.434) and 65% in culture-negative (Kappa 0.124) samples. It is worth noting that samples were split for testing by Xpert MTB/RIF and FluoroType MTB, which can affect the results due to uneven distribution of bacilli throughout a sample. However, homogenisation and vortexing prior to splitting was undertaken in order to minimise this risk.

A likely source of the high number of FluoroType-positive tests in culture-negative patients was the manual sample preparation, which is prone to cross-contamination in high-volume settings. Although this could be largely prevented using the GenoXtract system for automated extraction, purified nucleic acid is still manually transferred from the GenoXtract and added to the reaction vessels, along with buffers. This provides an opportunity for contamination that is not present in fully automated/integrated closed systems, such as GeneXpert, which had a higher specificity in this study.

TABLE 1 Diagnostic accuracy of FluoroType MTB stratified by light-emitting diode fluorescence smear microscopy smear microscopy positivity, HIV status and Xpert MTB/RIF result

	Culture positive (n=70)		Culture negative (n=226)		All	Sensitivity	Specificity
	FluoroType MTB positive	FluoroType MTB negative	FluoroType MTB positive	FluoroType MTB negative			
Overall	62	8	91	135	296	88.6% (78.7–94.9%)	59.7% (53–66.2%)
Smear positive	50	0	11	6	67	100% (92.9–100%)	35.3% (14.2–61.7%)
Smear negative	12	8	80	129	229	60.0% (36.1–80.9%)	61.7% (54.8–68.3%)
HIV positive	21	4	44	50	119	84.0% (63.9–95.5%)	53.2% (42.6–63.6%)
HIV negative	33	0	27	46	106	100% (89.4–100%)	63.0% (50.9–74.0%)
HIV unknown	8	4	20	39	71	66.7% (34.9–90.1%)	66.1% (52.6–77.9%)
Xpert MTB/RIF positive	53	2	13	1	69	96.4% (87.5–99.6%)	7.1% (0.2–33.9%)
Xpert MTB/RIF negative	9	6	78	134	227	60.0% (32.3–83.7%)	63.2% (56.3–69.7%)

Data in parentheses are 95% confidence intervals.

The study therefore found FluoroType MTB has a sensitivity equivalent to other molecular tests and identified more culture-positive samples than Xpert MTB/RIF, but its specificity was lower than expected, probably due to DNA contamination during the sample preparation steps rather than an inherently low specificity in the assay. Although this risk of contamination would be greatly reduced in laboratories with well-developed capacity for molecular techniques, it can prove challenging in settings without this capacity. Laboratories in high-burden countries considering its use should use the FluoroType MTB system together with GenoXtract. Furthermore, upgrading the current FluoroType MTB system to a fully automated, possibly closed system, like Xpert MTB/RIF should be considered by the producer.

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Received: Oct 07 2016 | Accepted after revision: March 11 2017

Support statement: The authors would like to acknowledge Hain Lifescience (Germany) for their provision of a FluoroCycler and sufficient FluoroType MTB assays to carry out the study. Hain Lifescience had no influence on the study design, data analysis or preparation of the manuscript. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: None declared.

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