Validation of PrimeStore Molecular Transport Medium® for PCR-based detection of *Mycobacterium tuberculosis* in sputum samples

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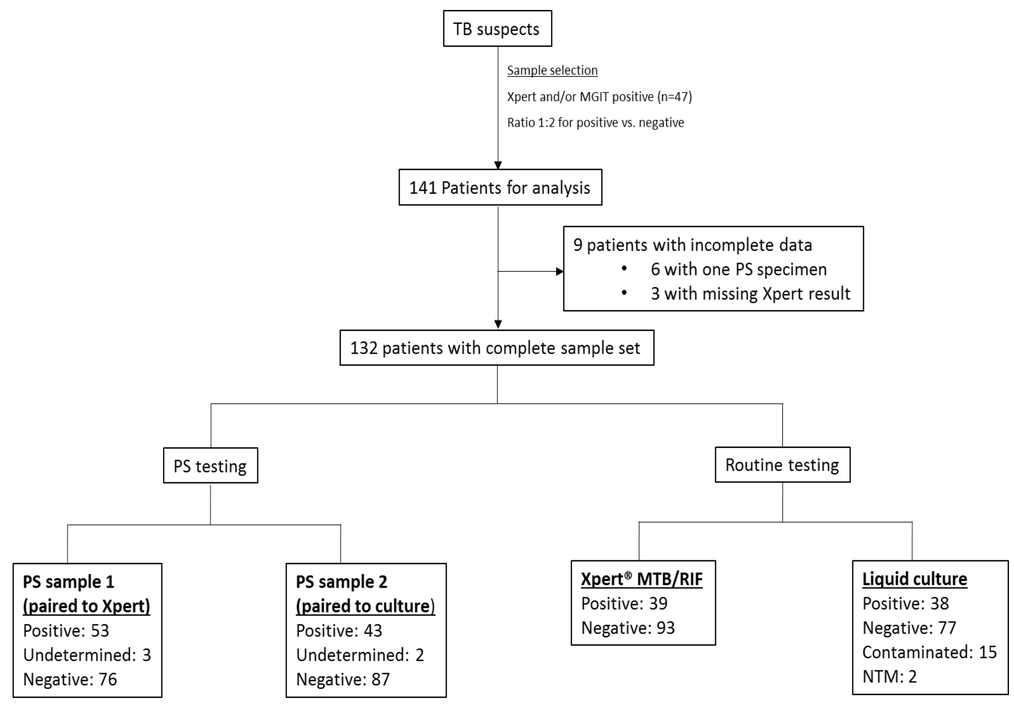
Introduction

- Molecular tests for *Mycobacterium tuberculosis* provide sensitive and rapid diagnosis of TB.
- Implementation of these tests in resource-poor settings has been limited due to logistic challenges, lack of skills, and higher cost.
- PrimeStore Molecular Transport Medium® (PS-MTM) preserves bacterial DNA and allows transport of sputum samples to a centralized facility for molecular testing.[1]
- We compare molecular testing of sputum samples collected in PS-MTM with smear microscopy and Xpert MTB/Rif® for diagnosis of TB in rural South Africa.

Figure 1: Flowchart of study



Material & methods

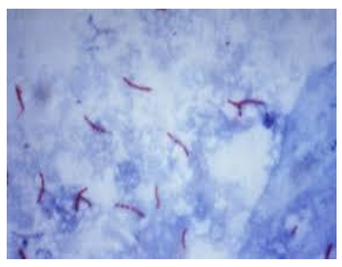
- Sputum samples were selected from a prospective study in which two specimens were collected from patients with cough ≥2 weeks (**Fig. 1**) at primary healthcare facilities in rural Mopani District, South Africa.[2]
- Shortly after expectoration and before processing by either Xpert® or Ziehl-Neelsen smear microscopy and liquid culture, about 100 µL of sputum was transferred from each specimen into PS-MTM tube using a flocked swab.
- Samples were transported at ambient temperature to the University of Pretoria where they were recoded and transported by air to a central laboratory in San Antonio, Texas, USA, for blinded molecular testing.
- DNA was extracted from 200 µL PS-MTM solution using PrimeXtract™ followed by PrimeMix® PCR (PM-PCR) for detection of *M. tuberculosis*.[3]

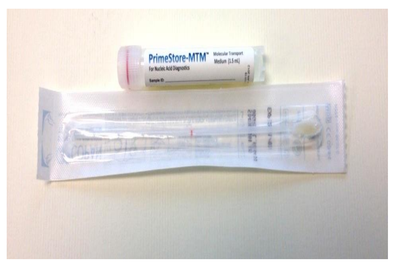
Results

- The 132 samples included in this analysis were positive by microscopy (n=23), Xpert (n=39), liquid culture (n=38) or PM-PCR (n=44); two samples had an indeterminate result in PM-PCR (C_T value 38-40).
- There was high concordance of PS-MTM/PM-PCR with positive result in smear microscopy (96%); another 22/107 (21%) of smear microscopy negative samples were PM-PCR positive (**Tab 1**).
- Concordance between PS-MTM/PM-PCR and positive Xpert result was 85% (33/36); another 11/91 (12%) Xpert negative samples tested positive in PM-PCR.
- Detection of *M. tuberculosis* by PS-MTM/PM-PCR was significantly more frequent than by microscopy (p<0.001) but similar to Xpert (p=0.33).

Table 1. PS-MTM/PM-PCR with smear microscopy, Xpert and culture for detection of Mycobacterium tuberculosis

	Smear microscopy		Xpert MTB/Rif		Liquid culture	
	Positive (n=23)	Negative (n=109)	Positive (n=39)	Negative (n=99)	Positive (n=38)	Negative (n=94)
PM-PCR Positive	22 (96%)	22 (20%)	33 (85%)	11 (12%)	31 (82%)	13 (14%)
PM-PCR Negative	1 (4.3%)	85 (78%)	6 (15%)	80 (86%)	7 (18%)	79 (84%)
PM-PCR Undetermined	0	2 (1.8%)	0	2 (2.2%)	0	2 (2.1%)





Discussion & conclusion

- Molecular tests of sputum samples from rural areas can successfully be performed at centralized laboratories after transport in PS-MTM.
- Such a diagnostic system would enhance detection of *Mycobacterium tuberculosis* by smear microscopy and could provide an alternative to laboratory-based Xpert as baseline test.
- An advantage of the PS-MTM/PM-PCR system is that only partial volume is used in the initial test allowing for further molecular testing if indicated.

References / Notes

1. Daum LT, Worthy SA, Yim KC, et al. A clinical specimen collection and transport medium for molecular diagnostic and genomic applications. *Epidemiol Infect* 2011; **139:** 1764 – 73.
2. Peters RP, Jonkman K, Brand J, et al. Cohort study of clinic- versus laboratory-based Xpert MTB/Rif for diagnosis of tuberculosis in South Africa. *Submitted for publication.*
3. Daum LT, Choi Y, Worthy SA, et al. A molecular transport medium for collection, inactivation, transport, and detection of Mycobacterium tuberculosis. *Int J Tuberc Lung Dis* 2014; **18:** 847-9.

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