Diagnosing Extra Pulmonary Tuberculosis using Xpert MTB/RIF: A Laboratory Algorithm

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Introduction

GeneXpert technology has a pooled sensitivity and specificity of 88% and 99% respectively for diagnosing pulmonary TB, but evidence (up to March 2012) for its use for diagnosing extra pulmonary (EP)TB is comparatively weak. This is due to small sample sizes, differences in pre-processing methodologies, input volumes and population variation across studies: sensitivity ranges of 25%- 97% and specificity 89% - 100%.
In 2011 the prevalence of pulmonary TB in South Africa was 768/100,000 (399-1,250), with an incidence of 993/100,000 (819-1,180): 325,321 new pulmonary TB cases and 47,285 (15%) new EPTB cases notified. **Management of EPTB disease therefore needs to play a more prominent role in the National Tuberculosis Control Programmes**, especially in high HIV burden settings.

Xpert MTB/RIF (Xpert) has been **nationally implemented in South Africa** throughout smear microscopy laboratories as a replacement for smear microscopy for initial diagnosis of pulmonary TB. The use of **Xpert for non-respiratory specimens TB diagnosis was investigated** with the aim of establishing the **most appropriate laboratory testing algorithm** for South Africa with the current knowledge base and availability.

Materials and Methods

Laboratory site and specimen receipt
The study was performed (August 2012 to February 2013) in the National Health Laboratory Mycobacteriology Referral Laboratory in Johannesburg, which provides a routine diagnostic service to public sector hospitals and clinics in 4 out of 6 districts of the Gauteng province of South Africa. Majority EPTB specimens are from hospitalized patients. Ethics clearance for the use of residual specimens for laboratory evaluations was approved (University of the Witwatersrand Human Ethics Committee (number M120875)).

Laboratory Processing of EPTB specimens.
- EPTB specimens contaminated with bacteria were identified or excluded by sheep blood agar platting and incubating at 37C for 24hrs.
- Non-sterile specimens were decontamination with NALC-NaOH.
- Fluid specimens >20mls were centrifuged before pellet inoculation into MGIT.
- Tissue and biopsy specimens were homogenised in 0.5ml phosphate buffer before MGIT inoculation.
- Auramine smear microscopy (if requested) was performed prior to MGIT.
- Growth of *Mycobacterium tuberculosis* complex was confirmed by Ziehl -Neelson (ZN).
  - Definitive identification of AFB samples was by GenoType MTBDR*plus*v1 (if DST requested),
  - by GenoType Mycobacterium CM line probe assay or by MPT64 antigen immunoassay (SD Bioline) if DST was not requested.
- If resistance detected by MTBDR*plus*v1 assay, further DST by MGIT proportions method was performed (Becton Dickinson).

- After routine processing and inoculation into MGIT, **residual specimen** (>0.5ml after decontamination; 1ml un-concentrated) was **tested by Xpert** as per the manufacturer's instructions.

Additional Xpert processing: 168 EPTB specimens with clear appearance were processed by Xpert without the pre-addition of sample reagent (SR) buffer, to determine if SR's harsh use on these specimens decreases the assay's performance.

Data Analysis performed by STATA v.12. Liquid culture was considered the reference standard for sensitivity and specificity calculations. This analysis focussed on all specimen types except those labelled as FNA, as this was previously reported from this region.

References

1. Boehme, C.C., et al., *Rapid molecular detection of tuberculosis and rifampin resistance.* N Engl J Med, 2010.; 2, WHO. *Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children: Policy Update.* http://www.stoptb.org/wg/gli/., 2013;

Results

The **total number of EPTB specimens** received for routine laboratory testing = **7916** (Table 1 (a). Number of **specimens with sufficient residual specimen volume for Xpert testing, = 1175** (Table 1(b)).
Residual specimens were from patients:
- Ranging in age from <1yr to 96yrs (median age of 39 yrs),
- 43 samples from children<15 yrs.
- 55% from males.
- **Only 3% of CSF specimens** had sufficient residual volume for Xpert testing.
- All 1175 specimens were plated on sheep agar plates,
  - 36 (3%) were contaminated with bacteria and followed NALC-NaOH processing.
  - This was not specific to any one specimen type, however 30.5% (11/36) were still contaminated on MGIT culture; Xpert testing was successful in all these.
- Overall 10.5% (124/1175) MGIT cultures were contaminated, with the majority (58%, 72/124) being fluids that were thick (86%: 62/72).
  - All (100%, 124/124) generated Xpert results of which 28% (35/124) were MTB positive.
- Xpert could not generate results in 0.5% (6/1175) due to 5011 errors (signal loss due to loss of tube pressure).

Table 1: List of EPTB specimens received and those with residual volume for Xpert testing.

Specimens type	(a) Frequency received in 6 months, n (%)	(b) Frequency with residual volume tested by Xpert, n (%)
Cerebrospinal fluid	2719 (34)	37 (3)
Fine needle aspriate (mostly lymph node)	2536 (32)	84 (7)*
Fluid (pleural, ascitic, other)	2008 (25)	890 (76)
Pus	417 (5)	119 (10)
Tissue biopsies	184 (2)	31 (3)
Dialysis fluid or urine	35 (0.4)	10 (1)
Scrapings	7 (0.1)	0
Bone	5 (0.1)	4 (0.3)
Stool	3 (0.1)	0
Catheter tip	2 (0.3)	0
total n	**7916**	**1175**

*only those labelled specifically as LNA (not as the majority labelled as FNA) where included in the analysis

Performance of the Xpert compared to MGIT culture

- Xpert positivity is 22% (260/1175) compared to 23.5% (277/1175) by MGIT,
- Overall Xpert sensitivity is 59% (CI 53, 65) and specificity is 92% (CI 90, 94).
- Fewer (7.6%) RIF resistant cases (10/132) were identified by traditional methods than Xpert which provided an early diagnosis of RIF resistance (9.6%) in 25/260 cases (100% specificity).

- Highest sensitivity is in pus, then aspirates and fluids (ascetic then pleural).
- Highest sensitivity is amongst thick specimens 87% (CI 76, 94), and significantly different to clear specimens 48% (CI 36, 61).
- This was unchanged with traces of blood 52% (CI 44, 60) or pre-centrifugation 57% (CI 28, 82) among clear specimens.
- Eliminating the harsh SR buffer from Xpert processing among clear specimens (n=168) did not improve the performance.
- Xpert (124 additional results) is less affected by contaminating bacteria than MGIT (10.5% contamination).
- The specificity of the Xpert was lower among pus and aspirates (76% and 78% respectively), but specificity of Xpert on pulmonary specimens is excellent (99%), and therefore likely these are true-positive cases.

Table 2: Xpert accuracy on EPTB specimen types >n=70 and based on specimen characteristic.

Xpert assay perfromance	MGIT culture	Xpert	Sensitivity (Culture Reference)	Specificity (Culture Reference)
Total sample number (n=1175)				
M.tb positive, n(%)	277 (23.5%)	260 (22%)		
M.tb negative, n(%)	774 (65.9%)	909 (77.4%)	n=1045	
Contaminated/ error, n(%)	124 (10.5%)	6 (0.5%)	59% (53, 65)	92% (90, 94)
Comparison for majority specimen types				
Specimen type Pus, n=119			n=92	
M.tb positive	43 (36%)	62 (52%)		
M.tb negative	49 (41%)	57 (48%)	91% (78, 97)	76% (60, 85)
Contaminated/ error	27 (23%)			
Specimen type Aspirate, n=84			n=75	
M.tb positive	n=20 (23.8)	n=30 (46%)		
M.tb negative	n=55 (65.5%)	n=54 (64%)		
Contaminated/ error	n=9 (10.7%)		80% (56, 94)	78% (65, 88)
Specimen type Fluid, n=890			n=812	
M.tb positive	206 (23%)	159 (18.3%)		
M.tb negative	612 (69%)	725 (81%)		
Contaminated/ error	72 (8%)	6 (0.7%)	51% (44, 58)	94% (92, 96)
Comparison amongst fluids				
Specimen type Pleural fluid, n= 528			n=482	
M.tb positive, n(%)	126 (24%)	92 (17%)		
M.tb negative, n(%)	362 (67%)	430 (81%)		
Contaminated/ error, n(%)	40 (8%)	6 (1%)	47% (38, 56)	94% (91, 96)
Specimen type Ascitic fluid, n=153			n=139	
M.tb positive, n(%)	32 (21%)	25 (16%)		
M.tb negative, n(%)	107 (70%)	128 (84%)		
Contaminated/ error, n(%)	14 (9%)		59% (41, 76)	97% (92, 99)
Specimen type Other fluid, n=209			n=191	
M.tb positive, n(%)	48 (23%)	42 (20%)		
M.tb negative, n(%)	143 (68%)	167 (80%)		
Contaminated/ error, n(%)	18 (9%)		54% (39, 69)	92% (87, 96)

XpertMTB/RIF performance by specimen appearance, n (%)		MGIT culture			Xpert MTB/RIF			Sensitivity (Culture Reference)	Specificity
	n=1175	Positive	Negative	Contaminated	Positive*	Negative	Error		
clear (watery), n(%)	292 (25%)	62	201	29	51	240	1	n=262: 48% (36, 61)	93% (89, 96)
bloody, n(%)	10 (0.9%)	0	9	1	1	9	0		
thick, n(%)	255 (22%)	62	131	62	92	162	1	n=192: 87% (76, 94)	85% (77, 90)
trace of blood, n(%)	618 (53%)	153	433	32	116	498	4	n=582: 52% (44, 60)	93% (91, 96)
Xpert performance on centrifuged specimens									
		Positive	Negative	Contaminated	Positive*	Negative	Error	Sensitivity	Specificity
Not centrifuged : n=1004 (85.5%)		241	673	90	206	792	6	n=908: 57% (50, 63)	94% (91, 95)
Centrifuged on receipt: n= 171 (14.6%)		36	101	34	54	117	0	n=137: 75% (58, 89)	81% (72,88)

Conclusion

1. **Xpert's** use for **diagnosing EPTB could significantly reduce labour in the laboratory and reduce the diagnostic delay**.
2. Xpert can be **introduced for TB screening of EPTB specimens and the pulmonary Xpert algorithm can be similarly followed** with confirmation by DST and its use in the context of clinical suspicion.
3. Implementation will require a full costing analysis and on-going studies for specific tissue types such as CSF (the predominant EPTB specimen received) where volumes and specimen preparation procedures have not been defined in this study.