JCM Accepts, published online ahead of print on 31 August 2011
J. Clin. Microbiol. doi:10.1128/JCM.01310-11

Xpert® MTB/RIF for the rapid diagnosis of tuberculous lymphadenitis from Fine Needle Aspiration biopsy specimens

Louis J Ligthelm[1*], Mark P Nicol[5] , Kim GP Hoek[2], Rachael Jacobson[5] , Paul D van Helden[3] , Ben J Marais[4], Robin M Warren[3], Colleen A Wright[1]

Affiliations

Divisions of [1]Anatomical Pathology and [2]Medical Microbiology of the Department of Pathology, , National Health Laboratory Service, Tygerberg Hospital and [3]DST/NRF Centre of Excellence for Biomedical Tuberculosis Research/ MRC Centre for Molecular and Cellular Biology Division of Molecular Biology and Human Genetics of the Department of Biomedical Sciences, Faculty of Health Sciences Stellenbosch University, South Africa. [4]Westmead Children's Hospital and Sydney Medical School, University of Sydney, Sydney, Australia [5]Division of Medical Microbiology, University of Cape Town and National Health Laboratory Service and Groote Schuur Hospital, South Africa.

***Corresponding Author:**

Dr. Louis J Ligthelm, Division of Anatomical Pathology, PO Box 19063, Tygerberg 7505, South Africa

Tel: -27-21-938 4041. Fax: -27-21-938 6559. Email: louisligthelm@sun.ac.za

Running Head: Xpert® MTB/RIF on FNAB of lymph nodes

Key words: tuberculosis, FNAB, Xpert® MTB/RIF

Conflicts of interest: None to declare

Word Count Abstract: 51

Word Count Text: 1019

ABSTRACT

This study demonstrates the excellent diagnostic accuracy of the Xpert® MTB/RIF test in patients with tuberculous lymphadenitis. The test sensitivity and specificity was 96.7% (95%CI, 86.6-100) and 88.9% (95%CI, 69.6-100) respectively, and correctly identified 6/6 (100%) of the cytology smear negative/culture positive cases and 1 of 2 (50%) rifampin resistant cases.

TEXT

Tuberculous lymphadenitis is the most common extrapulmonary manifestation of TB (4,8), and the majority of cases have no active lung involvement. Fine needle aspiration biopsy (FNAB) offers a feasible and safe option for specimen collection (11,15). The use of cytology together with the confirmation of acid-fastness by Ziehl-Neelsen staining and Papanicolaou stain-induced fluorescent microscopy as well as mycobacterial detection by culture offers excellent yields (13,14), but remains limited by the absence of species confirmation, slow turn-around times and/or lack of drug resistance guidance. Conventional microbiological culture and drug susceptibility testing are not always available and in rare instances may take 6 weeks or longer (10).

The World Health Organization (WHO) endorsed Xpert® MTB/RIF combines sample processing and real time polymerase chain reaction (PCR) in a fully automated platform and detects *Mycobacterium tuberculosis* complex and rifampin resistance in less than 2 hours (2,3,9). Xpert® MTB/RIF has been used successfully on various extrapulmonary specimens including urine and stool (6), but has not been rigorously evaluated with the use of tissue specimens such as FNAB.

To determine the diagnostic utility of the Xpert® MTB/RIF, FNAB were collected from 50 consenting patients by aspirating two passes of a 23- or 25-g needle attached to a 10ml syringe (IRB 05/03/043). Two smears were prepared from each aspirate, one fixed with commercial cytology fixative for Papanicolaou staining and evaluation by fluorescent microscopy and the other air dried for Giemsa and subsequent ZN staining. Smears were

evaluated for adequacy and for a morphological diagnosis and cases were excluded from the analysis if either one or both passes had inadequate cellular material on smears. Both ZN and Papanicolaou stain-induced fluorescent microscopy evaluations were performed for direct detection of mycobacteria on all specimens. Residual material from one of the aspirates was rinsed in a Mycobacterial Growth Indicator Tube (MGIT 960, Becton Dickinson, USA) by aspirating a small volume of fluid into the syringe and expressing it back into the MGIT 960 tube, followed by incubation in a MGIT 960 instrument for mycobacterial culture. Positive cultures were identified as *Mycobacterium tuberculosis* complex and genotypic drug susceptibility testing was done using Genotype MTBDR*plus* assay (Hain Lifesciences, Germany) (1).

The residual material from the remaining aspirate was rinsed as above into 0.7 ml sterile phosphate buffered saline (PBS) in a 10ml headspace glass vial sealed with a TFE/Sil Septa and Aluminum open top seal. Sample preparation buffer was then added to the vial in a 2:1 ratio, incubated at room temperature and subsequently processed for Xpert® MTB/RIF testing as previously described (2).

Performance calculations, including test sensitivity, specificity and predictive values were done using Statistica version 8 to compare the diagnostic performance of the Xpert® MTB/RIF test to the reference standard (as positive cytology (cytomorphology consistent with mycobacterial infection *and* direct visualization of the organism on ZN and/or Papanicolaou stain-induced fluorescent microscopy) and/or culture positive for *M. tuberculosis* (11,12)).

Of the 50 patients recruited, 48 cases had adequate smears for diagnosis (see Figure 1 for patient recruitment flow diagram). In total, cytomorphological features associated with TB were seen in 32 (66.7%) patients, non-specific reactive nodes identified in 10 (20.8%), acute bacterial lymphadenitis in 1 (2.1%), malignancy in 4 (8.3%) epithelial inclusion cyst in 1 (2.1%) (Table 1).

Compared to the reference standard, Xpert® MTB/RIF correctly identified 29 out of 30 TB cases (sensitivity 96.7%, 95%CI, 86.6-100) (Table 2). The possible "false negative" result had a prolonged transit interval of 9 days before Xpert® MTB/RIF testing, which may have affected the result. Xpert® MTB/RIF was positive in two cases with negative cytomorphology and culture (specificity 88.9%, 95%CI, 69.6-100). The cytomorphology from one of the "false positive" results showed a necrotizing suppurative lymphadenitis, which is consistent with TB. However, no organisms could be identified on microscopy or culture. The cytomorphology of the other false positive result showed an epithelial inclusion cyst, and the reason for this false positive result remains unknown. One case had a negative culture result with positive cytology (including mycobacterial identification) and a positive Xpert® MTB/RIF test. This patient had been on TB treatment for one month at the time of specimen collection, which provides the likely explanation for this discrepant result.

The Xpert® MTB/RIF test was positive in all 6 smear negative culture positive cases and correctly identified the 1 of the 2 rifampin resistant cases. The average time to result for

103 microbiological culture was 18.5 days (range 9-55 days), while the Xpert® MTB/RIF test
104 result was available within 2 hours of commencing the test. This represents a substantial
105 reduction in diagnostic delay, thereby permitting real-time decision making and planning
106 of treatment (5).
107
108 A recent study by Hillemann *et al.* demonstrated the effectiveness of Xpert® MTB/RIF on
109 extrapulmonary tissue (6). In that study, the combined sensitivity and specificity of
110 77.3% and 98.2% was reported, respectively. Our study is the first to evaluate the
111 performance of Xpert® MTB/RIF in diagnosing tuberculous lymphadenitis through the
112 use of FNAB specimens. Study limitations include the small number of rifampin
113 resistant cases identified and the fact that the research was conducted in a referral center,
114 as ideally the technique is suited to use in peripheral laboratories to be effective in
115 controlling the disease. A positive aspect of the study is that the patient population is
116 representative of patients presenting with peripheral lymphadenopathy in most TB/HIV
117 endemic areas. It is unlikely that our patient cohort had exacerbated disease as compared
118 to patients presenting at primary health-care clinics as these patients are routinely referred
119 from the primary health-care clinic to the referral centre for FNAB.
120
121 In conclusion, FNAB is a simple procedure which can be performed in an outpatient
122 setting by clinicians or nursing staff after a short training period (7,15). It is ideal for use
123 in resource-limited settings, including more remote and rural areas (15). Specimen
124 collection is simple and safe. With the use of a transport vial virtually no sample
125 preparation is required and there is minimal risk of contamination. Furthermore, the

transmission risk to the operator may also be reduced. Combining FNAB and rapid genotypic diagnosis using automated systems should greatly improve access to appropriate diagnosis and treatment for patients with tuberculous lymphadenitis.

130 **ACKNOWLEDGMENTS**

131 We thank Mr. Justin Harvey (Stellenbosch University) for statistical assistance.

132 **REFERENCES**

133

134 1. **Barnard, M., H. Albert, G. Coetzee, R. O'Brien, and M. E. Bosman**. 2008.
135 Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume
136 public health laboratory in South Africa. Am.J.Respir.Crit Care Med. **177**:787-792.

137 2. **Boehme, C. C., P. Nabeta, D. Hillemann, M. P. Nicol, S. Shenai, F. Krapp, J.**
138 **Allen, R. Tahirli, R. Blakemore, R. Rustomjee, A. Milovic, M. Jones, S. M.**
139 **O'Brien, D. H. Persing, S. Ruesch-Gerdes, E. Gotuzzo, C. Rodrigues, D.**
140 **Alland, and M. D. Perkins**. 2010. Rapid molecular detection of tuberculosis and
141 rifampin resistance. N.Engl.J.Med. **363**:1005-1015.

142 3. **Boehme, C. C., M. P. Nicol, P. Nabeta, J. S. Michael, E. Gotuzzo, R. Tahirli, M.**
143 **T. Gler, R. Blakemore, W. Worodria, C. Gray, L. Huang, T. Caceres, R.**
144 **Mehdiyev, L. Raymond, A. Whitelaw, K. Sagadevan, H. Alexander, H. Albert,**
145 **F. Cobelens, H. Cox, D. Alland, and M. D. Perkins**. 2011. Feasibility, diagnostic
146 accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for
147 diagnosis of tuberculosis and multidrug resistance: a multicentre implementation
148 study. Lancet **377**:1495-1505.

149 4. **Golden, M. P. and H. R. Vikram**. 2005. Extrapulmonary tuberculosis: an
150 overview. Am.Fam.Physician **72**:1761-1768.

151 5. **Helb, D., M. Jones, E. Story, C. Boehme, E. Wallace, K. Ho, J. Kop, M. R.**
152 **Owens, R. Rodgers, P. Banada, H. Safi, R. Blakemore, N. T. Lan, E. C. Jones-**

Lopez, M. Levi, M. Burday, I. Ayakaka, R. D. Mugerwa, B. McMillan, E. Winn-Deen, L. Christel, P. Dailey, M. D. Perkins, D. H. Persing, and D. Alland. 2010. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J.Clin.Microbiol. **48**:229-237.

6. **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.

7. **Leiman, G.** 1995. The Registered Nurse as Aspirator: Experience in a Busy Cytopathology Unit. Acta Cytol. **39**:1010.

8. **Marais, B. J., R. P. Gie, H. S. Schaaf, A. C. Hesseling, D. A. Enarson, and N. Beyers**. 2006. The spectrum of disease in children treated for tuberculosis in a highly endemic area. Int.J.Tuberc.Lung Dis. **10**:732-738.

9. WHO. WHO endorses new rapid tuberculosis test. 2010. Available at: http://www.who.int/tb/features_archive/new_rapid_test/en/ Accessed 08-12-2010.

10. WHO. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. 2008. Available at http://whqlibdoc.who.int/hq/2008/WHO_HTM_TB_2008.392_eng.pdf Accessed 08-09-2009.

11. **Wright, C. A., A. C. Hesseling, C. Bamford, S. M. Burgess, R. Warren, and B. J. Marais**. 2009. Fine-needle aspiration biopsy: a first-line diagnostic procedure in

paediatric tuberculosis suspects with peripheral lymphadenopathy? Int.J.Tuberc.Lung Dis. **13**:1373-1379.

12. **Wright, C. A., K. G. Hoek, B. J. Marais, H. P. van, and R. M. Warren**. 2010. Combining fine-needle aspiration biopsy (FNAB) and high-resolution melt analysis to reduce diagnostic delay in Mycobacterial lymphadenitis. Diagn.Cytopathol. **38**:482-488.

13. **Wright, C. A., B. M. van der, D. Geiger, J. G. Noordzij, S. M. Burgess, and B. J. Marais**. 2008. Diagnosing mycobacterial lymphadenitis in children using fine needle aspiration biopsy: cytomorphology, ZN staining and autofluorescence -- making more of less. Diagn.Cytopathol. **36**:245-251.

14. **Wright, C. A., Z. Y. van, S. M. Burgess, L. Blumberg, and G. Leiman**. 2004. Mycobacterial autofluorescence in Papanicolaou-stained lymph node aspirates: a glimmer in the dark? Diagn.Cytopathol. **30**:257-260.

15. **Wright, C. A., R. M. Warren, and B. J. Marais**. 2009. Fine needle aspiration biopsy: an undervalued diagnostic modality in paediatric mycobacterial disease. Int.J.Tuberc.Lung Dis. **13**:1467-1475.

190 **Table 1: Demographics and diagnostic outcome of patients referred for fine**
191 **needle aspiration biopsy with possible mycobacterial lymphadenitis.**
192

	Number (N=48)	Percent
Age		
<5 years	2	4.2
5-20 years	6	12.5
>20 years	40	83.3
Gender		
Male	20	41.7
Female	28	58.3
Patients on TB treatment	2	4.2
HIV infection status		
Positive	9	18.8
Negative	3	6.6
Unknown	36	75
Culture +/HIV +	4/9	44.4
Cytological features		
Reactive lymph node	10	20.8
Features consistent with TB	32	66.7
Acute bacterial infection	1	2.1
Malignant or suggestive of malignancy	4	8.3
Epithelial inclusion cyst	1	2.1

Cases with cytomorphology suggestive of TB		
Smear +[a], Culture +, GeneXpert® +	22	45.8
Smear +[a], Culture +, GeneXpert® –	1	2.1
Smear –, Culture +, GeneXpert® +	6	12.5
Smear +[a], Culture –, GeneXpert® +	1	2.1
Smear –, Culture –, GeneXpert® +	1	2.1
Smear –, Culture –, GeneXpert® –	1	2.1

193

194 [a] Smear + = Cytomorphology suggestive of TB with direct visualization of organism.

195 + positive, – negative

196

Table 2: Diagnostic accuracy of the Xpert® MTB/RIF test versus various reference standards.

Reference standard	Sensitivity		Specificity		Predictive values	
	n/N (%)	95% CI	n/N (%)	95% CI	PPV (%)	NPV (%)
Xpert® vs. Reference Standard*	29/30 (96.6)	86.6-100	16/18 (88.9)	69.6-100	93.5	94.1
Xpert® vs. Culture	28/29(96.6)	86.1-100	16/19(84.2)	63.6-100	90.3	94.1
Xpert® vs. smear– Culture+	6/6(100)	100	(100)	100	100	100
Autofluorescence vs. Culture	22/29(75.9)	60.3-91.4	18/19(94.7)	79.3-100	95.7	72
ZN vs. Culture	12/29(41.4)	23.5-59.3	19/19	100	100	52.8

*Reference standard as defined in the text – positive cytology (cytomorphology consistent with mycobacterial infection with direct visualization of the organism) and/or mycobacterial culture. ZN = Ziehl-Neelsen stain; n = index group; N = control group

204 **LEGENDS**

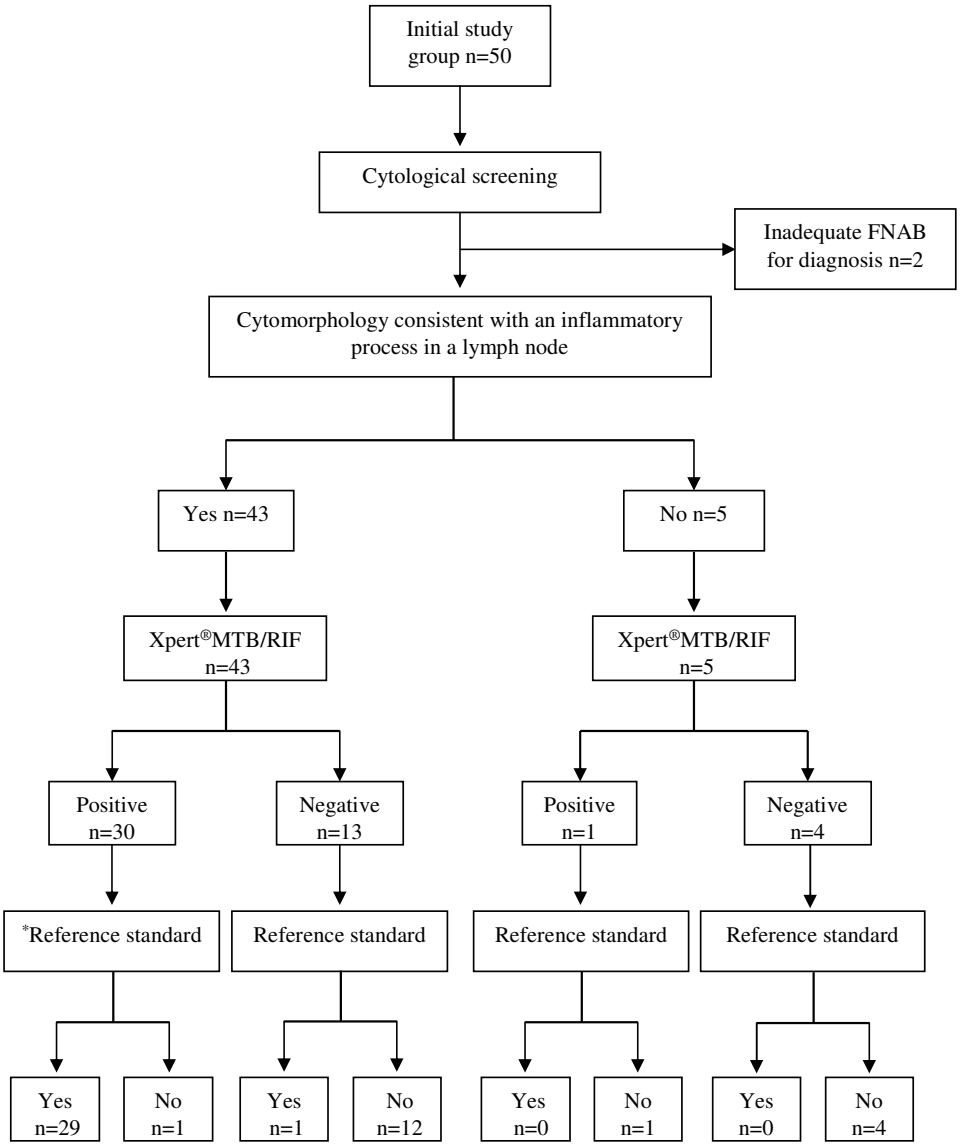
205

206 **Figure 1.** Flow diagram of all patients referred for fine needle aspiration biopsy with

207 possible mycobacterial lymphadenitis.

208

Figure 1



* Reference standard = Cytomorphology suggestive of TB with direct visualization of the organism and/or bacteriological culture
FNAB = Fine Needle Aspiration Biopsy