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## DIAGNOSTIC ACCURACY OF XPERT® MTB / RIF COMPARED TO MICROSCOPY-BASED METHODS FOR DIAGNOSING TUBERCULOUS LYMPHADENITIS FROM FINE NEEDLE ASPIRATES AT THE PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA

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Running title: Diagnostic accuracy of Xpert assay.

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*Running title: Diagnostic accuracy of Xpert assay.*

**ABSTRACT:**

Data on the accuracy of Xpert® MTB/RIF (Xpert) assay in detecting TB in lymph node aspirates in Papua New Guinea (PNG) is scanty. This study evaluated Xpert performance in diagnosing tuberculous lymphadenitis (TBLN) using lymph node needle aspirates at the Port Moresby General Hospital (PMGH). The objective of the study was to compare Xpert accuracy to acid fast bacilli (AFB) microscopy, cytomorphology, a composite reference test (CRS) and culture. A total of 107 eligible subjects were recruited out of 1080 clinic attendees. Results showed Xpert detected significantly more cases of TBLN than AFB microscopy (66 vs 35; p=0.001). Compared to AFB microscopy Xpert had a sensitivity of 45.4% (95% CI 33.1-58.1), specificity of 87.8% (95% CI 73.8-95.9), positive predictive value (PPV) of 85.7% (95% CI 71.6-93.4) and negative predictive value (NPV) of 50.0%% (95% CI 43.8-56.1). There was no difference between Xpert and cytomorphology (66 vs 60; p=0.5). Compared to cytomorphology Xpert had a sensitivity of 71.6% (95% CI 58.5-82.5), specificity of 51.1% (95% CI 35.7-66.3), PPV of 66.1% (95% CI 58.2-73.2) and NPV of 57.5% (95% CI 45.2-68.9). There was no difference between Xpert and CRS (66 vs 71; p=0.6). Compared to CRS Xpert had a sensitivity of 76.0% (95% CI 64.4-85.3), specificity of 66.6% (95% CI 49.0-81.4), PPV of 81.8% (95% CI 73.5-87.9) and NPV of 58.4% (95% CI 46.7-69.4). Culture was completed on 24 subjects with positive isolates in 14 giving a culture yield of 58.3%. Of the 24 subjects, Xpert was positive in 21 subjects. There was no difference between Xpert and culture (21 vs 14; p=0.8). Compared to culture Xpert had a sensitivity of 100.0% (95% CI 76.8-100.0), specificity of 30.0% (95% CI 6.6-65.2), PPV of 66.6% (95% CI 57.1-75) and NPV of 100.0%. The results suggest Xpert is more sensitive than AFB microscopy but comparable to cytomorphology and CRS for TBLN diagnosis in the PNG context. Xpert can be used for diagnosing TBLN at PMGH.

**Keywords**: Extrapulmonary tuberculosis, lymph node aspirate, acid fast bacilli microscopy

INTRODUCTION:

Sensitivity and specificity of microscopy are variable with the form of extra-pulmonary TB (EPTB) [1]. Although Papua New Guinea (PNG) national guidelines recommend microscopy of fine needle aspirates (FNA) for laboratory confirmation of tuberculous lymphadenitis (TBLN), in practice diagnosis is made based on clinical features. This may be due to inadequate laboratory infrastructure, shortage of pathologists and trained medical laboratory personals to do cytology microscopy [2,3,4].

Microscopic diagnosis of TBLN at Port Moresby General Hospital (PMGH) is based on identification of cytomorphological features of granulomatous inflammation (epitheloid cells, caseous necrosis, granulomas) using May-Grunwald-Giemsa (MGG) stain with or without stainable acid-fast bacilli (AFB) by Ziehl-Neelsen (ZN) stain. Xpert ® MTB/RIF (Xpert) testing for mycobacterium tuberculosis (MTB) complex is available in 22 public health facilities throughout PNG and used for testing respiratory specimens. World Health Organisation (WHO) published guidelines for Xpert testing of non-respiratory samples but these recommendations are conditional and the test has to be evaluated against local settings for optimum use [5,6].

Denkinger et al [1] conducted a systemic review and meta-analysis of 18 studies that evaluated Xpert diagnostic accuracy against culture from tissues of FNA specimens in detecting TB in lymph node and found Xpert to have sensitivity range between 50% and 100% [1]. When Xpert was evaluated against a composite reference standard for TBLN pooled sensitivity was 81.2% [1]. Xpert was also found to perform better than existing methods of diagnosing EPTB using other samples such as pleural fluid or cerebrospinal fluid [1]. As a result, WHO recommends Xpert over conventional tests for TB diagnosis in lymph node and other non-respiratory specimens to exclude TB in a very sick child [1]. With the objective of nationwide implementation of Xpert testing of FNA aspirates, this pilot study at PMGH was done to evaluate the diagnostic performance of Xpert (index test) with existing microscopy-based methods (reference tests) of TBLN diagnosis.

METHODOLOGY:

Study population, setting and sampling procedure:
Port Moresby General Hospital is the largest and only tertiary referral hospital in PNG with approximately 600 beds.

The Pathology Department conducts a twice weekly FNA clinic that processes a maximum of 30 patients per clinic day. Patients that were clinically suspected of TBLN by the treating physicians referred for FNA between November 2014 and August 2015 were recruited via the weekly FNA clinics. Every third consecutive patient meeting the eligible criteria was invited to participate in the study. Both inpatient and outpatient attendees were recruited. Each patient was clinically examined and interviewed using a standardised questionnaire piloted before the study implementation.

Ethical approval was obtained from the Medical Research Advisory Council of Papua New Guinea

National Department of Health (MRAC file No: 54-6-2, November 11, 2014).

Inclusion criteria:

Patients with lymph node enlargement of the cervical, axillary and inguinal regions with a clinical suspicion of TBLN were included. Both male and female patients in all age groups were eligible for the study. Inclusion criteria for culture were (1) rifampicin resistance detection by Xpert and (2) study sample selected for external quality assurance testing. External quality assurance sample selection was done per standard operating procedure for MTB at the PNG National Department (NDoH) Central Public Laboratory (CPHL) housed within PMGH. Cultures were done at the Queensland Mycobacterium Reference Laboratory (QMRL), Brisbane, Australia.

Lymph node sampling and processing:

Nodes of more than two centimetres were sampled using aspiration and nodes less than two centimetres were sampled using non-aspiration technique. A 22 or 23-gauge hypodermic needle attached to a 10ml syringe was used with the aspiration technique. A 2ml vacuum pressure was created in the syringe after inserting the needle into the chosen sampling site and the needle moved back and forth using a rapid steady motion without completely withdrawing the needle.

Sampling was stopped when sample (blood, pus or mixture of both) was visible at the hub of the needle. In the non-aspiration method, a 22 or 23-gauge needle was passed into the enlarged gland without the syringe and sampling was by moving the needle back and forth using a rapid steady motion without completely withdrawing the needle. Aspiration was drawn into the needle by capillary action. One or two passes were done for each study subject. Two smears were made for each study subject. One slide was stained with MGG stain and the other with ZN stain. Remainder of the sample in the needle was rinsed with 2ml physiological saline in a sterile container. The saline-aspirate mixture was sent to CPHL and used in the Xpert assay. Samples testing positive for rifampicin resistance on Xpert or if selected as part of CPHL external quality program were sent to QMRL in Brisbane Australia for culture. Standard personal protection equipment was worn and biosafety procedures were followed at all times.

Index and reference tests:

Xpert was defined as the index test. The reference tests were (1) AFB microscopy, (2) cytomorphology (epithelioid cells, granulomas, caseous necrosis), (3) composite reference standard (CRS) comprising AFB and cytomorphology and (4) culture. Positive AFB was defined as stainable AFB by ZN stain and positive cytomorphology by MGG was defined as presence of cytomorphological features of granulomatous inflammation. Positive CRS was defined as positive AFB and/or positive cytomorphology. Positive Xpert was defined as MTB detection (positive or borderline positive).

Data were tabulated in Microsoft Excel sheet and transferred to SPSS® for analysis. Demographic and clinical characteristics were recorded to describe the study population. Accuracy of the index test was described using sensitivity, specificity, positive predictive value (PPV) and

negative predictive value (NPV) using 2x2 tables. T-test was used for statistical significance testing between index test and reference tests with level of significance set at p value less than 0.05.

**RESULTS**:

Total of 1080 patients attended the FNA clinic during the study period and 107 consenting eligible subjects were recruited for the study. The mean age was 26 (±14) years. There were 45 (42.1%) males and 62 (57.9%) females with a male to female ratio of 0.7. Xpert detected MTB in 66/107 (61.6%) subjects. Acid fast bacilli was positive in 35/107 (32.7%) subjects, cytomorphology showed 60/107 (56.1%) positive cases and CRS was positive in 71/107 (66.3%) subjects (Table 1.0).

Xpert compared with AFB microscopy as the reference test showed Xpert detected significantly more MTB than AFB microscopy (66 positive cases versus 35 positive cases; p=0.001). Compared to AFB microscopy Xpert had a sensitivity of 45.4% (95% CI 33.1-58.1), specificity of 87.8% (95% CI 73.8-95.9), PPV of 85.7% (95% CI 71.6-93.4) and NPV of 50.0% (95% CI 43.8-56.1) (Table 2.0).

There was no difference between Xpert and cytomorphology (66 positive cases versus 60 positive cases; p=0.5). The sensitivity, specificity,

PPV and NPV of Xpert using cytomorphology as reference test were 71.6% (95% CI 58.5-82.5), 51.1% (95% CI 35.7-66.3), 66.1% (95% CI 58.2-73.2) and 57.5% (95% CI 45.2-68.9) respectively (Table 2.0).

There was no difference between Xpert and CRS as the reference test (66 positive cases versus 71 positive cases; p=0.6). Compared to CRS Xpert had a sensitivity of 76.0% (95% CI 64.4-85.3) and specificity of 66.6% (95% CI 49-81.4). The PPV and NPV of Xpert were 81.8% (95% CI 73.5-87.9) and 58.5% (95% CI 46.7-69.4) respectively (Table 2.0).

Culture was completed on 24 subjects with positive isolates in 14/24 (58.3%) cases (Table 3.0). Xpert detected MTB in all culture positive samples (14/14, 100.0%). Of the culture negative samples Xpert was positive in 7/10 (70.0%).

Sensitivity and specificity of Xpert using culture as reference test were 100.0% (95% CI 76.8-100) and 30.0% (95% CI 6.6-65.2) respectively. The PPV was 66.6% (95% CI 57.1-75) and NPV was 100.0%. There was no difference between Xpert (21/24, 87.5%) and culture (14/24, 58.3% [21 versus 14; p=0.8]). Of the 14 isolates, nine (9/14; 64.2%) were resistant to at least one drug tested. Six of the nine resistant isolates (6/9; 66.6%) were MDR-TB. Mono-resistant rate was 33.3% (3/9).

Table 1: AFB microscopy, cytomorphology, Xpert, culture and CRS test results

|          | AFB    | Cytomorphology | Xpert  | Culture* | CRS**  |
|----------|--------|----------------|--------|----------|--------|
| Positive | 35/107 | 60/107         | 66/107 | 14/24    | 71/107 |
| Negative | 72/107 | 47/107         | 41/107 | 10/24    | 36/107 |

*Only 24 samples were sent for culture. **CRS = composite reference standard.

Table 2. Diagnostic accuracy of Xpert compared with microscopy-based methods for TBLN diagnosis at PMGH.

| Xpert vs various reference tests | Sensitivity (%) | | Specificity (%) | | Predictive value (%) | |
| --- | --- | --- | --- | --- | --- | --- |
| | (n**/N***) | 95% CI | (n**/N***) | 95% CI | Positive (95% CI) | Negative (95% CI) |
| Xpert vs AFB | 45.4 (30/35) | 33.1-58.1 | 87.8 (36/72) | 73.8-95.9 | 85.7 (71.6-93.4) | 50.0 (43.8-56.1) |
| Xpert vs cytomorphology | 71.6 (44/60) | 58.5-82.5 | 51.1 (23/45) | 35.7-66.3 | 66.1 (58.2-73.2) | 57.5 (45.2-68.9) |
| Xpert vs CRS* | 76.0 (54/71) | 64.4-85.3 | 66.6 (24/36) | 49.0-81.4 | 81.8 (73.5-87.9) | 58.5 (46.7-69.4) |
| Xpert vs culture | 100.0 (14/14) | 76.8-100.0 | 30.0 (3/10) | 6.6-65.2 | 66.6 (57.1-75.0) | 100.0 |

*CRS = composite reference standard, composite of positive cytomorphology and AFB microscopy. **n = positive cases using Xpert. ***N = positive cases using respective reference tests.

Table 3: Drug susceptibility test results of resistant isolates (Total isolates = 14, Total resistant isolates = 9)

| Drugs Tested | Resistant rate |
| --- | --- |
| Amikacin | 0 |
| Streptomycin | 0 |
| Isoniazid 0.1 mg/L | 2/9 (33.3%) |
| Isoniazid 0.4 mg/L | 6/9 (66.6%) |
| Ofloxacin | 1/9 (11.1%) |
| Rifampicin | 9/9 (100%) |
| Ethambutol | 4/9 (44.4%) |
| Pyrazinamide | 2/9 (22.2%) |
| Ethionamide | 3/9 (33.3%) |
| Kanamycin | 0 |
| Capreomycin | 0 |
| Cycloserine | 0 |
| *PAS (para-aminosalicylic acid) | 0 |

**DISCUSSION**:

Sensitivity of Xpert in our study ranged between 45.4% and 100% varying with the reference test. Xpert had 100% sensitivity with culture as reference test, compared to CRS Xpert had 76% sensitivity, 71.6% sensitivity compared to cytomorphology and 45.4% sensitivity with AFB microscopy. Other studies evaluating Xpert in diagnosing TBLN have used other composite reference tests or culture as the reference tests and obtained sensitivities ranging between 59% and 96.1% [7,8,9,10,11,12]. The sensitivity of Xpert is also variable with gross appearance of the aspirate with sensitivities between 73% and 87% where presence of pus was associated with a higher sensitivity rate [8,9,10,11,12].

The specificity of Xpert in this study ranged between 30% and 87.8% varying with the reference test. Xpert had specificity of 87.8% compared to AFB microscopy, 66.6% specificity with CRS as reference test, cytomorphology 51.1% specificity and compared to culture Xpert specificity was 30%. Our specificity results are lower than similar studies that reported specificities between 88.9% and 100% [6,8,9,10,11,12]. The difference in specificities between these studies and our results are most likely due to the limited number of aspirates sent for culture in our study. Of the 10 culture negative cases, seven were positive on Xpert of which six had cytomorphological features of TBLN and two were AFB positive.

Laboratory confirmation of TBLN at PMGH is based on AFB microscopy and identifying cytological features of granulomatous inflammation. These methods are labour intensive, require trained cytologists and have long result turn-around time. This practice is the same in most resource limited settings [4,14,15,16,17]. Although microscopy of FNA is cheap and suitable in resource limited settings, cytomorphological features are non-specific and lack sensitivity without demonstration of AFB [18,19,20]. Presence of caseous necrosis and neutrophils is associated with high AFB positivity [14]. There is also positive association between pus aspirates, granulomas, presence of neutrophils or necrosis and AFB detection and it has been suggested that AFB must be demonstrated and other causes of granulomatous inflammation be excluded before making a diagnosis of TBLN [18,19,20]. In PNG where there is shortage of pathologists, laboratory medical scientists can be trained to do needle aspiration of enlarged lymph nodes and process FNA aspirates for Xpert diagnosis of TBLN. This may improve result turn-around time allowing earlier commencement of TB treatment. Xpert also has the advantage of detecting drug resistant TB and allows clinicians to consider second line drugs while culture results are pending, particularly in sick children where obtaining respiratory samples is challenging. Fine needle aspirate smear and cytological microscopy for TBLN diagnosis in PNG is limited to pathologists but the shortage of pathologists hinders nationwide implementation of FNA cytology for TBLN diagnosis. Although Xpert is currently available in 22 sites in PNG making it possible to diagnose TBLN in health facilities that has no resident pathologist, a cheaper alternative

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would be to train medical laboratory scientist in PNG to perform FNA cytomorphological analysis for diagnosis of TBLN.

This study reports a mycobacterial culture yield of 58.3% (14/24). Culture yield of MTB from FNA is reported to be between 42% and 83% [13]. Positive HIV status is also associated with a higher yield from FNA aspirates in adults [13]. The present study's design did not permit gathering information of the HIV status of subjects. Factors contributing to negative culture results may have included inadequate volume (one patient) or TB treatment for more than two weeks (two patients). Although physiological saline was used for emulsifying the FNA prior to shipment for culture, the yield is higher in MTB specific transport mediums [13]. Prolonged storage (more than 10 days at CPHL) of specimen prior to shipment to Australia may have resulted in reduced number of viable bacilli negatively affecting culture results as suggested by some studies [6].

Studies on multi-drug resistant TB (MDR-TB) in PNG have reported rates of 4.6% and 26% [21,22]. A large population-based survey in PNG has shown that the national MDR-TB rate is 2.7% in new cases and 19.1% in previously treated cases [23]. Of the 14 isolates, nine (9/14; 64.2%) were resistant isolates showing resistance to at least one drug with rifampicin mono-resistant rate of 33.3% (3/9) and MDR-TB rate of 66.6% (6/9). The high rate is preliminary and indicates the need for a larger sample size study looking at the resistant pattern of MTB isolates from subjects with TBLN at PMGH. The present study's drug susceptibility test results are consistent with Aia et al [23] who showed that MTB drug resistance in PNG is heterogenous [23].

The MDR-TB isolates showed resistance to rifampicin (9/9, 100%), ethambutol (4/9, 44.4%) and pyrazinamide (n=2, 14%). This pattern is similar to other published data on MTB drug resistance in PNG [21,22,24]. Whereas other studies in PNG reported isolates showing resistance to streptomycin, capreomycin and para-aminosalicylic acid [21,22], isolates in this study did not show resistance to these drugs. The observed differences may be due to different strains causing TBLN as suggested by a study in Ethiopia [25].

The use of Xpert in PNG is limited to testing respiratory specimens with the exception of cerebrospinal fluid [2]. The results of the present study provided evidence to re-evaluate this recommendation in PNG and contributed to the development of a laboratory algorithm for processing of FNA specimens. The newly developed laboratory algorithm has been included in the revised PNG national guidelines for the testing of EPTB specimens. This study further demonstrates that Xpert processing of FNA aspirates can be tailored to local settings in other high TB-burden countries.

There are limitations to our study. Although culture is the preferred reference standard, only 24 samples were sent for culture and reflect the realities of the study setting. We did follow up cases to determine clinical outcome. A follow up prospective study that includes monitoring of clinical outcomes can help show impact of Xpert on the management of drug resistant TBLN in PNG. The use of 2ml physiological saline may be inadequate and or may not be the ideal transport medium for

MTB culture. A MTB specific solution may have produced higher culture yields.

**CONCLUSIONS**:

The results suggest Xpert is comparable to cytomorphology and a microscopy-based composite reference test (CRS) for TBLN diagnosis at PMGH. The sensitivity and specificity of Xpert compared to existing microscopy methods for diagnosing TBLN is acceptable in the PNG context. Most importantly, Xpert can be implemented nationally to provide laboratory confirmation of TBLN and offer the added advantage of detecting MDR-TB, particularly in children.

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