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Gene Xpert Assay for the Diagnosis of Tuberculous Lymphadenitis on Concentrated Fine Needle Aspirates in a Tertiary Care Hospital in Chamba District

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Abstract
Introduction: *The diagnosis of tuberculous lymphadenitis remains challenging.The routinely used methods have sub-optimal sensitivity.Recently,WHO recommends GeneXpert to be used as the initial diagnostic test in patients suspected of having extra-pulmonary tuberculosis.However,this was a conditional recommendation.In this study we evaluated the performance of Xpert for the diagnosis of TBL on concentrated fine needle aspirates (FNA) in Chamba.*
Methods: *FNA was collected from presumptive TBL cases. Two smears were prepared from each aspirate and processed for cytology and conventional microscopy The concentrated sediment was used for culture and Xpert test.Composite bacteriological methods (culture and/or smear microscopy) were considered as a reference standard.*
Result: *Out of 143 enrolled suspects, 64.3% (92/143) were confirmed TBL cases. Xpert detected M. tuberculosis complex (MTBC) in 60.1% (86/143) of the presumptive TBL cases. The sensitivity of Xpert compared to CRS was 87.8% and specificity 91.1% . The sensitivity was 27.8% for smear microscopy and 80% for cytology compared to CRS. Xpert was positive in 4 out of 45 culture- and smear-negative cases. Among 47 cytomorphologically non-TBL cases, 15 were positive on Xpert Resistance to rifampicin was identified in 4.7% of Xpert-positive cases.*
Conclusion: *Xpert test showed a high sensitivity and specificity for the diagnosis of TBL on concentrated FNA samples.*

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I. Introduction

Tuberculosis (TB) remains a major public health problem in India. India ranks Ist in the list of the high TB burden countries. In India extra pulmonary tuberculosis comprises 20% of all TB cases[1].Accurate diagnosis and early treatment of TB has the potential to reduce morbidity and mortality associated with TB lymphadenitis (TBL). However, the differential diagnosis of TBL is broad and laboratory confirmation is of paramount importance to guide appropriate therapy[2,3]. Cytology and conventional smear microscopy have been used as the initial diagnostic tools for TBL in resource poor settings [3,4]. Fine needle aspiration cytology is a simple and rapid diagnostic technique, but with low specificity because of the presence of similar cytologic indicators in lesions other than those associated with TB [5,6].Conventional smear microscopy lacks sensitivity due to the paucibacillary nature of fine needle aspirates(FNA) [7]. Mycobacteriological culture and drug susceptibility testing are not always available in resource poor settings like Chamba District. In line with these limitations more rapid and reli- able methods are needed.InDecember2010,WHO endorsed GeneXpertMTB/RIF1 for use in TB laboratories. The Xpert assay consists of a closed system that is based on real-time polymerase chain reaction (PCR). It can be used by operators with minimal technical expertise, enabling the diagnosis of TB and simultaneous detection of rifampicin resistance within 2 hours [8]. The Xpert assay has been validated and optimized for sputum samples to diagnose HIV- associated TB and multidrug-resistant TB. WHO strongly recommends widespread use of Xpert for these groups of patients[9,10].More recently a number of studies were done to evaluate this assay using non-respiratory clinical samples from patients suspected of having Extra Pulmonary TB [11,12].In 2014, WHO has recommended Xpert over the conventional tests(including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having Extra Pulmonary TB[13].However, this was a conditional recommendation due to very low-quality evidence available. More

studies are therefore needed particularly in settings with high EPTB prevalence. Thus, we evaluated the performance of Xpert for the diagnosis of TBL using routinely collected FNA samples and compared it against cytology, smear microscopy and culture.

II. Materials and Methods

Ethical clearance was first obtained from ethical review board. All patients or guardians in case of children were requested for written consent prior to enrolment to the study. Any information concerning the patients was kept confidential. Laboratory results were reported back to the physicians for treatment initiation or decision as early as available. This study was conducted at Pandit Jawahar Lal Nehru Govt. medical college, Chamba, Himachal Pradesh. A total of 143consecutive outpatients clinically suspected of TBL and referred by attending clinicians for TB testing were enrolled in this study. Participants' demographic and clinical information were collected using a pre-tested questionnaire. The FNA sample, at least 1ml, was collected by a Pathologist in the Pathology diagnostic unit. Gross specimen appearance (caseous, purulent, and/or blood stained) was recorded at the time of specimen collection .The first few drops of the aspirates were used for cytomorphological diagnosis. Air dried smears were stained with Giemsa stain and examined by a pathologist. The cytological criteria for the diagnosis of TBL are based on the presence of the following cytomorphological appearances: epithelioid cell aggregate with or without Langerhans giant cells and necrosis, epithelioid cell aggregate without necrosis, necrosis without epithelioid cell aggregate or polymorphonucleocytes with necrosis [14].TB treatment was initiated based on the cytomorphological diagnosis. The remaining sample was processed for smear microscopy, culture and Xpert in the District TB Center,Chamba. Two drops from each specimen were used to make a smear for standard Ziehl-Neelsen (ZN)staining. Stained smears were examined for the presence of AFB under oil-immersion (100x)using a light microscope. Mycobacterial culture was done on Löwenstein-Jensen (LJ) medium within 2days of specimen collection. All FNA specimens were processed by the standard N-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) method with a final NaOH concentration of 1%. An equal volume of standard NALC-NALC/NaOH solution was added to the specimen and incubated for 15minutes. After neutralization by phosphate buffered saline (PBS) and centrifugation (15 minutes at 3000g), the sediment was resuspended in 1ml of sterile PBS. Finally 200 µl of sediment was used to inoculate on two LJ slants. The laboratory strain, M. tuberculosis H37Rv(ATCC27294) ,was used as a positive control. Random slants of LJ medium were inoculated with sterile distilled water in each run as negative controls. Culture positive results were confirmed for MTBC by Capilia TB-Neo test(TAUNS,Izunokuni, Japan.

Xpert test was performed using same aspirate done from fine needle aspiration.The sample reagent (1.5ml)supplied with the test was added in a 3:1 ratio to the sample sediment(0.5ml).The mixture was vortexed and incubated at room temperature for 15minutes.Two ml of the reagent sample mix was then transferred to an Xpert cartridge using a Pasteur pipette and the cartridge was loaded onto Xpert (Cepheid,DxSystem Version 4.0c)machine. Results were reported as positive or negative for M.tuberculosis. Rifampicin resistance results were reported as susceptible, resistant or indeterminate. Data were double entered and analysed using the SPSS software package (version 16).Sensitivity, specificity were calculated using composite bacteriological methods(Culture for M.tuberculosis onLJ)
GeneXpertMTB/RIFAssay for Diagnosis of Tuberculous Lymphadenitis medium and/or smear microscopy using ZNmethod)as a reference standard.

III. Results

A total of 143 patients with clinical presumptive TB presenting with lymphadenopathy were enrolled between Jan-June 2017. Out of these, 18.9% (27/143) were positive for TBL on smear microscopy, 60.1% (86/143) on Xpert and 61.5% (88/143) on culture. On cytological examination, 67.1% (96/143) had cytomorphological features suggesting TBL. Overall , 64.3% (92/143) of tested cases were positive for TBL by culture and/or smear microscopy (23 smear/ culture-positive,65culture-positive/smear-negative, 3 smear-positive/culture-negative and 1 smear-positive/culture contaminated) (Fig).The Xpert result was invalid for 1.4% (2/143) of tests performed. Patients demographic and lymph node characteristics are shown in Table 1-3. Smear microscopy detected AFB in 26% (23/88) of culture-positive and 6% (3/49) of culture-negative cases. Of five contaminated samples on culture, one was positive on smear microscopy. Culture was positive in74% (71/96) of cases with suggestive cytomorphology of TB and in 36.2% (17/47) of non-TBL suggestive cases. When compared to Culture, smear microscopy had 27.8% sensitivity and 100% specificity whereas cytology showed sensitivity of 80.0% and specificity of 57.8%. Xpert was positive for M. tuberculosis in 86.4%(76/88) of culture positive and 14.3% (7/49) of culture-negative cases. M.tuberculosis DNA was detected by Xpert in 3 out of 5 samples with contaminated cultures .Only one isolate was identified as NTM by the Capilia test and Xpert result was negative. MTBC isolates resistant to rifampicin were identified in4.7% (4/86) of Xpert positive cases. Rifampicin resistance status for one MTBC positive sample was indeterminate.

Xpert showed an overall sensitivity of 87.8%and specificity of91%. Xpert yielded a positive result in 56 out of 65smear-negative/culture-positive cases. Only two specimens that were reported as "scanty AFB" in smear microscopy were negative by Xpert. When the culture positive results are stratified by AFB smear results, the sensitivity of Xpert was 91% (20/22) in smear-positive and 86.2% (56/65) in smear-negative cases. A summary of the diagnostic accuracy of cytology and Xpert test compared to CRS is presented in Table 5.

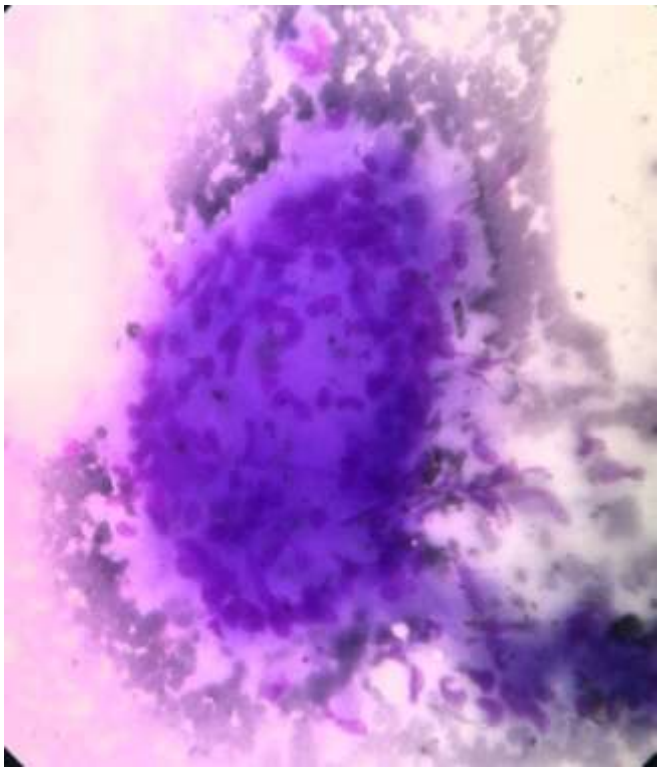
The cytomorphological features consistent withTB were observed in 67% (96/143) of the patients with lymphadenitis .The other diagnoses reported on cytology were chronic inflammation in 11.2% (16/143), suppurative abscess in 10.5% (15/143), reactive lymphadenitis in7.7% (11/143) and malignancy in 3.5% (5/143). Xpert detected M.tuberculosis in74% (71/96) of cases with suggestive cytomorphology of TB. In addition, Xpert was also positive in15cases with negative cytology:10 were from suppurative abscesses. In all these latter cases, TBL was confirmed by the CRS (Table 5). Gross lymph node aspirate was described as purulent in 51%(73/143) ,caseous in 40.6% (58/143) and bloodstained in 8.4% (12/143) of the cases. Xpert positivity rate was highest in aspirates with caseous appearance (69% (40/58)), and lowest in blood stained aspirates (41.7% (5/12)), although these differences were statistically not significant (Table 6).

Table 1: Showing the sex distribution of total enrolled patients:

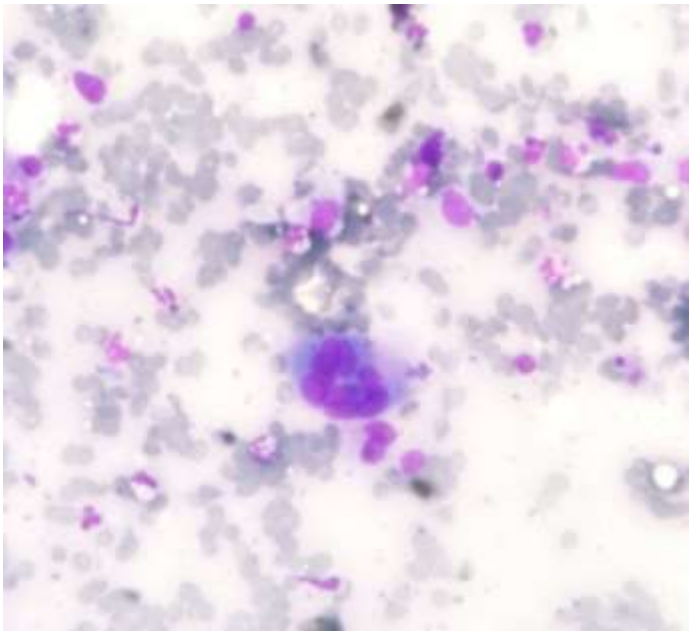
S.no	Sex	Number
1.	Male	67 (46.9%)
2.	Female	76(53.1%)

Table 2: Showing the age distribution of patients:

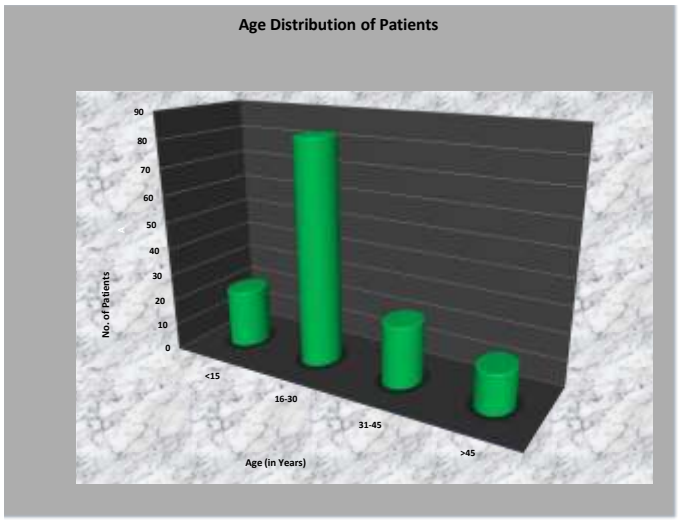
S.No	Age (in years)	No.of patients
1.	<15	22 (15.4%)
2.	16-30	83(58%)
3.	31-45	23(16.1%)
4.	>45	15(10.5%)

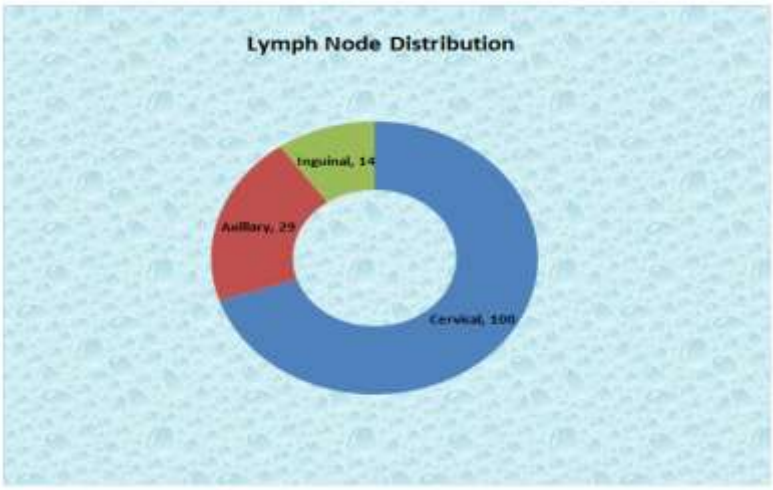


Epithelioid cell granuloma on MGG stain, 40 X. (Cytology)



Langhan's Giant cell , MGG stain, 40 X (Cytology





Langhan's Giant cell , MGG stain, 40 X (Cytology

Table 3: Showing lymph node distribution

S.No	Lymph Node	Distribution of L.node in patients
1.	Cervical	100 (69.9%)
2.	Axillary	29 (20.3%)
3.	Inguinal	14(9.8%)

Table 4: Showing specimen appearance

S.No	Specimen appearance	% distribution
1.	Purulent	73(50.1%)
2.	Caseous	58(40.6%)
3.	Bloody stained	12(8.4%)

Table 5: Comparisons of microscopic cytomorphological features and FNA gross appearance with Xpert test and (culture and/or ZN)

S.No	FNA Cytology Result	No.of patients(n)	Xpert positive patients(n/N)	Culture positive (n/N)
1.	Tuberculosis lymphadenitis	96	71/96 (74%)	74/96 (77%)
2.	Chronic Inflammation	16	3/16 (18.8%)	7/16 (43.8%)
3.	Suppurative Abscess	15	10/15 (66.7%)	10/15 (66.7%)
4.	Reactive Lymphadenitis	11	1/11 (9%)	0
5.	Malignancy	05	1/5 (20%)	1/5 (20%)

Table 6: Showing Gross FNA appearance

S.No	Gross FNA appearance	No. of patients	Xpert positive
1.	Purulent	73	41/73(56.2%)
2.	Caseous	58	40/58(69%)
3.	Bloody stained	12	5/12(41.7%)

IV. Discussion

The WHO strongly recommend the use of Xpert for the initial diagnosis of individuals suspected of MDR-TB or HIV associated TB [10].Based on very low quality evidence, WHO also conditionally recommends Xpert to be used rather than conventional methods as the initial diagnostic test in patients suspected of having EPTB[10,13].In Chamba, whereTB and MDR-TB are highly prevalent, the effectiveness of Xpert for diagnosing TBL and/or detection of drug resistance has not been conclusively demonstrated. In the present study, the sensitivity of Xpert was 87.8%. A systematic review and meta-analyses conducted by Denkinger et al showed that Xpert test has a sensitivity ranging from 50% to 100% with pooled sensitivityof 83%[15]More recently ,Penz et al reviewed 36 studies in their meta-analyses and confirmed Xpert pooled sensitivity of 87%that is similar to our study[16]. However, the sensitivityof Xpert in thecurrent study is lower than what was found in similar study by Ligthelm et al(sensitivity, 96.7%) [17].There were 11culture-positive cases which were negative on Xpert. The reason for false-negative Xpert test results may be due to the limited number of bacilli in the FNA sample or prolonged storage (median (IQR) delay of41(30– 45)days)of sample before Xpert testing. The specificity(91%)of the Xpert in the current study was found to be consistent with previous studies reported by others(specificity,89–99%) [15,16,17],but higher than the study done by Biadigilegn et al (specificity, 69.2%) [11].Seven culture-negative cases were Xpert positive. Five of these were positive for TBL on cytology and 3 on smear microscopy, suggesting the presence of nonviable bacilli due to either the harsh decontamination process or the nature of the caseous lesion in the lymph node tissue which may have contained dead tubercle bacilli. Such cases (Xpert-positive but culture-negative) are likely to be true TB positives as corroborated by the high specificity [12] and by the fact that the procedure is less prone to contamination due to the closed reaction chamber(real-time PCR technology) of Xpert. Even though conventional ZN microscopy has played an important role in the diagnosis of TBL in resource poor settings, Xpert detected MTB in 86%(56/65) of cases missed by smear microscopy. Only 2 smear-and culture-positive samples were negative by Xpert. In agreement with other studies[17],Xpert has a higher sensitivity than smear microscopy.There was little difference in the sensitivity of Xpert in smear-positive and smear-negative TBL cases. Xpert detected a significant proportion of smear-negative and culture-positive cases and significantly increased the relative proportion of diagnosed TBL cases. In developing countries ,smear microscopy is the only widely implemented method for quantifying the bacterial burden at the time of the initial diagnosis [18].Xpert provides a semi-quantitative measurement of the number of MTBC bacilli present in a clinical sample.. FNA cytology as an inexpensive and reliable tool for TBL has been studied by a number of investigators [3,19,20].It is one of the most commonly used methods in resource poor settings. In the current study the sensitivity of cytology was

comparable to that of Xpert,but the specificity was lower (57.8%), yielding many false positives. This may be due to non-specific cytomorphological features seen in cytology. On the other hand, cytomorphological features associated with suppurative abscess did not reliably exclude TBL in our study, which maybe explained by the absence ofcharacteristic features such as scattered epithelioid cells among the polymorphous population of lymphoid cells- indeed, our findings suggest that 'suppurative' features should be considered as suggestive of TB as the cause of the lymphadenitis. Xpert test offers rapid detection of rifampicin resistant MTBCstrains directly from the clinical sample, an important advantage over cytology and smear microscopy. Previous studies reported 98– 100% agreement in detection of rifampicin resistance strains using the Xpert test and phenotypic drug susceptibility test[11,12,21].In the current study, rifampicin resistance was identified in4.7% (4/86) of Xpert-positive cases. Two of these were retreatment cases. Our study has some limitations. Mycobacterial culture on LJ medium and/or smear microscopy was used as a reference standard though both of these methods are not sufficient to detect all TBL cases. Among culture and/or smear-negative cases there may be false negatives that started anti-TB treatment on clinical grounds and improved cases that were most likely true TB. Unfortunately we did not include clinical outcomes in our dataset. Thus, further prospective studies are required to evaluate the performance of Xpert on unprocessed fresh FNA samples by using a more sensitive liquid culture and/or histology as a reference standard or by adding clinical diagnosis (with response to treatment) to the standards. Moreover, while we only identified one NTM in culture, we did not speciate it, and our study is unable to reflect on the contribution of NTMs known to cause lymphadenitis, such as conclusion, our findings indicate that Xpert MTB/RIFtest is a useful tool for the M. scrofulaceum, M. avium complex, and M.kansasii. In detection ofMTBC with high sensitivity and specificity on concentrated fine needle aspirate with superior performance as compared to cytology and smear microscopy. Besides improved sensitivity, the Xpert was able to identify patients with TBL due to rifampicin resistant TB. The Xpert test is an easy and suitable method to be used in TB endemic settings and its implementation could significantly improve the rapid diagnosis of TBL.

V. Conclusion

Xpert test showed a high sensitivity and specificity for the diagnosis of TBL on concentrated FN samples . In addition, Xpert offered rapid detection of rifampicin-resistant M. Tuberculosis strains from lymph node aspirates.

References

[1]. Arora VK, Chopra KK-Extra pulmonary Tuberculosis. Indian J Tuber 2007;54:165-7.

[2]. Kurabachew M, EngerØ, SandaaR-A , Skuce R, BjorvatnB (2004) A multiplex polymerase chain reaction assay for genus-,group- and species-specific detection of mycobacteria. Diagnostic microbiology and infectious disease 49:99–104. PMID:15183858

[3]. Wright CA, vander Burg M, Geiger D, Noordzij JG, Burgess SM,et al.(2008) Diagnosing mycobacterial lymphadenitis in children using fine needle aspiration biopsy : cytomorphology, ZN staining and autofluorescence—making more of less. Diagnostic cytopathology 36:245–51.doi:10.1002/dc.20788 PMID:18335554

[4]. Kidane D ,Olobo JO, Habte A ,Negesse Y,Aseffa A ,et al. (2002) Identification of the causative organism of tuberculous lymphadenitis in Ethiopia by PCR.Journal of clinical microbiology 40:4230–4. PMID:12409403

[5]. Ammari FF, Hani AHB, Ghariebeh KI (2003) Tuberculosis of the lymph glands of the neck :alimited role forsurgery.Otolaryngology—Head and Neck Surgery 128:576–80.PMID:12707664

[6]. Boeree M, Kamenya A ,Liomba G, Ngwira B, Subramanyam V ,et al. (2014) Tuberculosis lymphadenitis ,a diagnostic problem in areas of high prevalence of HIV and tuberculosis .Malawi Medical Journal 11:56–9.

[7]. Annam V, Karigoudar MH, YelikarBR (2009) Improved microscopical detection of acid-fast bacilli by the modified bleach method in lymph node aspirates. Indian Journal of Pathology and Microbiology 52: 349.doi:10.4103/0377-4929.54991 PMID:19679958

[8]. World Health Organization (2011) Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational 'How-to';practical considerations.

[9]. World Health Organization (2010) Strategic and Technical Advisory Group for Tuberculosis (STAG- TB).Report of the Tenth Meeting. Geneva: WHO.

[10]. World Health Organization(2013) Automated Real-Time Nucleic Acid AmplificationTechnology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update. Geneva: WHO.

[11]. Biadglegne F ,Mulu A,Rodloff AC, Sack U (2014) Diagnostic performance of the Xpert MTB/RIF assay for tuberculous lymphadenitis on fine needle aspirates from Ethiopia.Tuberculosis.

[12]. Hillemann D,Rüsch-Gerdes S,Boehme C,Richter E (2011) Rapid molecular detection of extra pulmonary tuberculosis by the automated Gene Xpert MTB/RIFsystem.Journal of clinical microbiology 49: 1202-05 .doi:10.1128/JCM.02268-10 PMID:21270230

[13]. World Health Organization(2014) Xpert MTB/RIF implementation manual:technical and operational 'how-to';practical considerations.

[14]. Gupta AK, Nayar M, Chandra M (1991) Critical appraisal of fine needle aspiration cytology in tuberculous lymphadenitis. Acta cytologica 36:391–4.

[15]. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, et al.(2014) Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis:a systematic review and meta-analysis.European RespiratoryJournal:erj00078-02014.

[16]. Penz E, Boffa J, Roberts D, Fisher D, Cooper R, et al.(2015) Diagnostic accuracy of the Xpert1 MTB/ RIF assay for extra-pulmonary tuberculosis :A meta-analysis. The International Journal of Tuberculosis and Lung Disease 19:278–284.doi:10.5588/ijtld.14.0262 PMID:25686134

[17]. Ligthelm LJ ,Nicol MP,Hoek KG,Jacobson R,vanHelden PD,et al.(2011) Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens .Journal of clinical microbiology 49:3967–70 .doi:10.1128/JCM.01310-11 PMID:21880965

[18]. Prevention CfDCa (2003)AFB microscopy: characteristics, strategies and interpretation. Atlanta: Centers for Disease Control and Prevention.
[19]. Das DK (2000) Fine-needle aspiration cytology in the diagnosis of tuberculous lesions. Lab Medicine 31:625–32.
[20]. Mistry Y, Ninama GL, Mistry K, Rajat R, Parmar R,et al.(2012) Efficacy of Fine Needle Aspiration Cytology, Ziehl-Neelsen Stain and Culture(Bactec) in Diagnosis of Tuberculosis Lymphadenitis. National Journal of Medical Research 2:77–80.
[21]. Boehme CC, Nabeta P, Hillemann D ,Nicol MP, Shenai S ,et al.(2010)Rapid molecular detection of tuberculosis and rifampin resistance. New England Journal of Medicine 363:1005–1015.doi:10.1056/ NEJMoa0907847 PMID:20825313

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