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Truelab Micro PCR In Diagnosis of Extrapulmonary Tuberculosis: Our Experience

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Abstract

Introduction: India carries about 25% burden of tuberculosis. It is estimated that about 15-20% of all TB cases are of extrapulmonary origin. Recently, molecular amplification methods such as PCR and RT PCR have attracted interest of laboratorians as the diagnosis can be made in a short time and treatment initiated at the proper time.The present study was undertaken to evaluate the role of Truelab Micro PCR (Molbio diagnostics) in diagnosis of EPTB. *Materials and Methods:* This was a retrospective study conducted between January 2014 to June 2016.Atotal of 447 patients suspected of having EPTB clinically were included in the study. Specimens were collected from patients, both male and female, from different sites like pus, body fluids, lymph node aspirates, CSF, semen, urine, menstrual blood and endometrial tissue etc.according to standard protocols. All the specimens were run on Truelab MicroPCR system(Molbio diagnostics) according to standard protocols. *Results:* A total of 447 patients data was used for this study. Maximum number of samples were endometrial tissue (59.9%) followed by lymph node aspirates (11.63%), pus (4.92%),urine (4.47%), pleural fluid & menstrual blood (3.57%) CSF (3.13%), Ascitic fluid (2.90%) Synovial fluid (1.1%), vitreous fluid and peritoneal fluid (0.89%) , BAL fluid (0.67%) . Out of these different extra pulmonary samples 19(4.25%) were positive by RT PCR and 95.75% were negative. Highest positivity rate was observed inpus (27.2%), followed by CSF (21.4%), menstrual blood & pleural fluid (6.25%), lymph node aspirates(5.76%), urine(5%) and 1.49% in endometrial tissue. *Conclusion:* Truelab Micro PCR is portable,easy to use and provides accurate diagnosis of disease like EPTB with a rapid turn around time. It does not require skilled manpower and elaborate infrastructure and can be easily installed in resource limited settings. A complete patient centric solution for patients suffering from EPTB is a necessity and rapid, accurate diagnosis with Truelab Micro PCR and prompt therapywill help control TB burden in India.

Keywords: EPTB; Real Time PCR; Molecular Methods; True Lab MicroPCR.

Introduction

Tuberculosis is a major global health problem and it is estimated that approximately 8.8 million new cases were reported in year 2012 out of which 1.3 million succumbed to the disease [1]. India carries about 25% burden of tuberculosis and it is estimated

that about one third of the total TB cases are left undiagnosed and hence untreated.There is no reliable data available from India about the burden of extrapulmonary TB, but it is estimated that about 15-20% of all TB cases are of extrapulmonary origin.

The clinical presentation of EPTB is varied, depending upon the site involved, hence many cases may be missed or there might be delay in diagnosis and treatment due to lack of standardized methods for diagnosing EPTB,unlike pulmonary TB. Moreover, the paucibacillary nature of EPTB hinders the diagnosis. Conventional methods like smear and culture have limited diagnostic value in diagnosis of EPTB as it lacks sensitivity and specificity. Recently, molecular amplification methods such as PCR and RT PCR have attracted interest of laboratorians as the diagnosis can be made in a short time and treatment initiated at the proper time.

The present study was undertaken to evaluate the role of Truelab MicroPCR (Molbio diagnostics) in diagnosis of EPTB.

Materials and Methods

This was a retrospective study conducted between January 2014 to June 2016.Atotal of 447 patients suspected of having EPTB clinically were included in the study. Specimens were collected from patients,both male and female,from different sites like pus, body fluids,lymph node aspirates, CSF, semen, urine, menstrual blood and endometrial tissue according to standard protocols. Specimens were subjected to direct smear microscopy for presence of acid fast bacilli when the quantity and quality of specimen permitted. Provisional diagnosis of EPTB was made on the basis of cytology, radiology and response to anti tubercular treatment when confirmatory histopathological diagnosis was not available. All the specimens were run on Truelab MicroPCR saystem (Molbio diagnostics) according to standard protocols.

Results

A total of 447 patients data was used for this study . The patients were divided into 0–20 years, 21-40, 41–60 , 61–80 and more than 80 years age group in both the sexes.

Maximum patients (76.2%) were in 21-40 years age group, followed by (10.7%) in 0-20 years age group, 9.17% in 41-60 years, 3.57% in 61-80 years and 0.22 % in more than 80 years age group 19.2% were males and 80.8% were females. Male female ratio was 0.23:1 with maximum number of female patients in 21-40 years age group (Table 1).

Maximum number of samples were endometrial tissue (59.9%) followed by lymph node aspirates(11.63%), pus (4.92%), urine (4.47%), pleural fluid & menstrual blood (3.57%) CSF (3.13%), Ascitic fluid (2.90%) Synovial fluid (1.1%), vitreous fluid and peritoneal fluid (0.89%), BAL fluid (0.67%), and other sites like semen,ovarian cyst fluid, fluid from Pouch of Douglas(0.44%)and 0.22%from cervical tissue, tissue from sinus tract and skin lesion (Table 2).

Out of these different extra pulmonary samples 19(4.25%) were positive by RT PCR. and 95.75% were negative. Highest positivity rate was observed in pus (27.2%), followed by CSF (21.4%) menstrual blood & pleural fluid (6.25%), lymph node aspirates(5.76%), urine(5%) and 1.49% in endometrial tissue(Table 3).

No positive result was observed in vitreous fluid BAL fluid, Ascitic, Synovial and peritoneal fluids and other sites like ovarian tissue, fluid from Pouch of Douglas etc (Table 4).

268 patients with endometrial tissue as sample were divided into less than 20, 21-30, 31-40, 41to50 and more than 50 years age group, out of which 63.4% patients where in 21-30 years age group followed by 30.9% in 31-40 years, 3.73% below 20 years, 1.49% in 41-50 years age and 0.37% in more than 50 years age group (Table 5).

Table 1: Showing demographic distribution of patients

Age in years	Male	Female	Total	Percentage
0 To 20	24	24	48	10.70%
21 to 40	31	310	341	76.20%
41 to 60	19	22	41	9.17%
61 to 80	11	5	16	3.57%
>80	1		1	0.22%
TOTAL	86	361	447	
Total Percentage	19.20%	80.80%	100%	

Graph 1

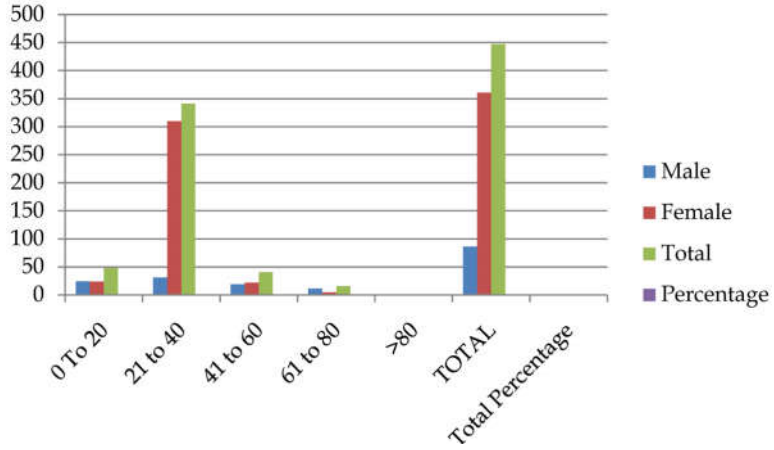


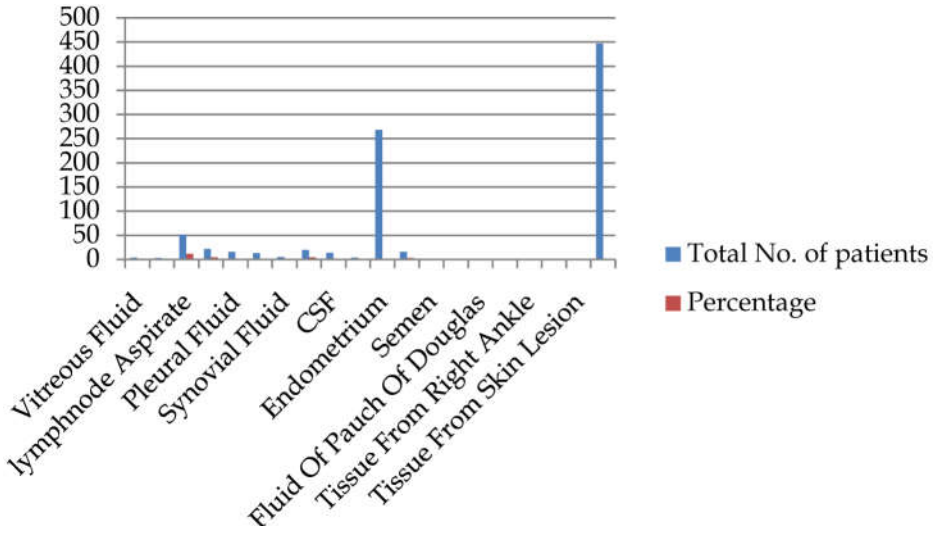
Table 2: Showing number of patients with different clinical specimens

Specimen site	Total No. of patients	Percentage
Vitreous Fluid	4	0.89%
Bal Fluid	3	0.67%
lymphnode Aspirate	52	11.63
Pus	22	4.92
Pleural Fluid	16	3.57%
Ascitic Fluid	13	2.90%
Synovial Fluid	5	1.11
Urine	20	4.47
CSF	14	3.13%
Peritoneal Fluid	4	0.89%
Endometrium	268	59.90%
Menstrual Blood	16	3.57
Semen	2	0.44
Ovarian Cyst Fluid	2	0.44
Fluid Of Pauch Of Douglas	2	0.44
Cervical Tissue	1	0.22
Tissue From Right Ankle	1	0.22
Tissue From Sinus Track	1	0.22
Tissue From Skin Lesion	1	0.22
Total	447	

Table 3: Showing number of positive patients with different clinical specimens

Specimen site	Total Positivepatients	Percentage
Vitreous Fluid	0	0.00%
Bal Fluid	0	0.00%
lymphnode Aspirate	3	5.76
Pus	6	27.2
Pleural Fluid	1	6.25%
Ascitic Fluid	0	0.00%
Synovial Fluid	0	0
Urine	1	5
CSF	3	21.4%
Peritoneal Fluid	0	0.00%
Endometrium	4	1.49%
Menstrual Blood	1	6.25
Semen	0	0.00%
Ovarian Cyst Fluid	0	0.00%
Fluid Of Pauch Of Douglas	0	0.00%
Cervical Tissue	0	0.00%
Tissue From Right Ankle	0	0.00%
Tissue From Sinus Track	0	0.00%
Tissue From Skin Lesion	0	0.00%
Total	447	4.25

Graph 2:



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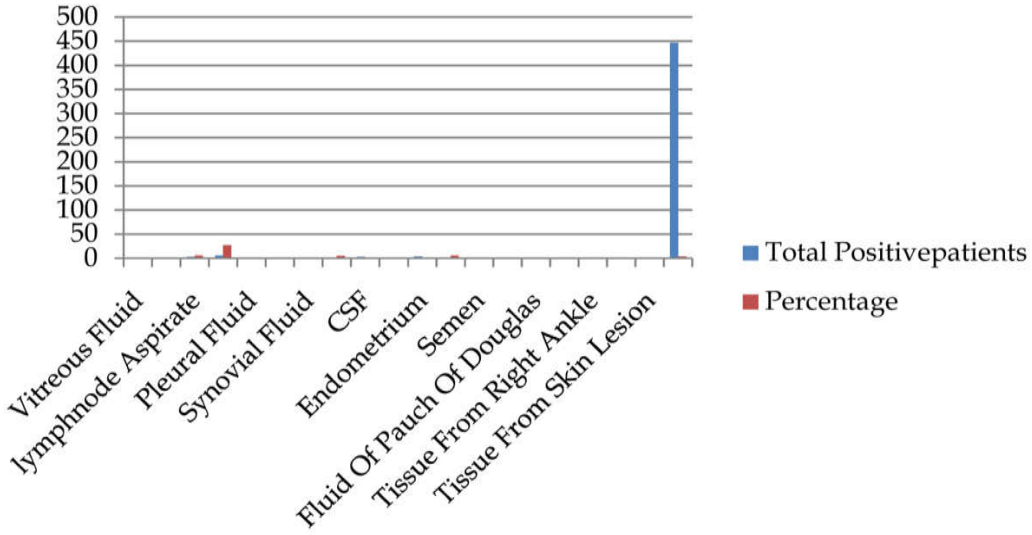


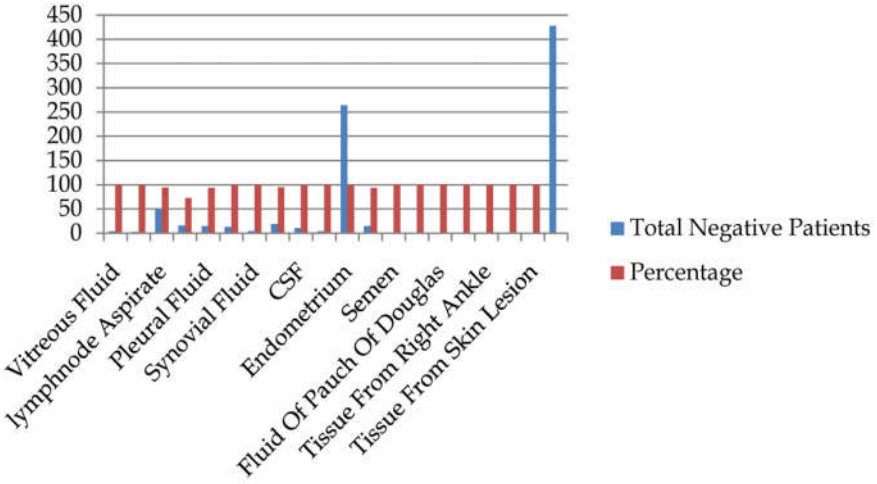
Table 4:

Specimen site	Total Negative Patients	Percentage
Vitreous Fluid	4	100
Bal Fluid	3	100
lymphnode Aspirate	49	94.24
Pus	16	72.8
Pleural Fluid	15	93.75
Ascitic Fluid	13	100
Synovial Fluid	5	100
Urine	19	95
CSF	11	78.6
Peritoneal Fluid	4	100
Endometrium	264	98.5
Menstrual Blood	15	93.75
Semen	2	100
Ovarian Cyst Fluid	2	100
Fluid Of Pauch Of Douglas	2	100
Cervical Tissue	1	100
ssue From Right Ankle	1	100
issue From Sinus Track	1	100
issue From Skin Lesion	1	100
Total	428	

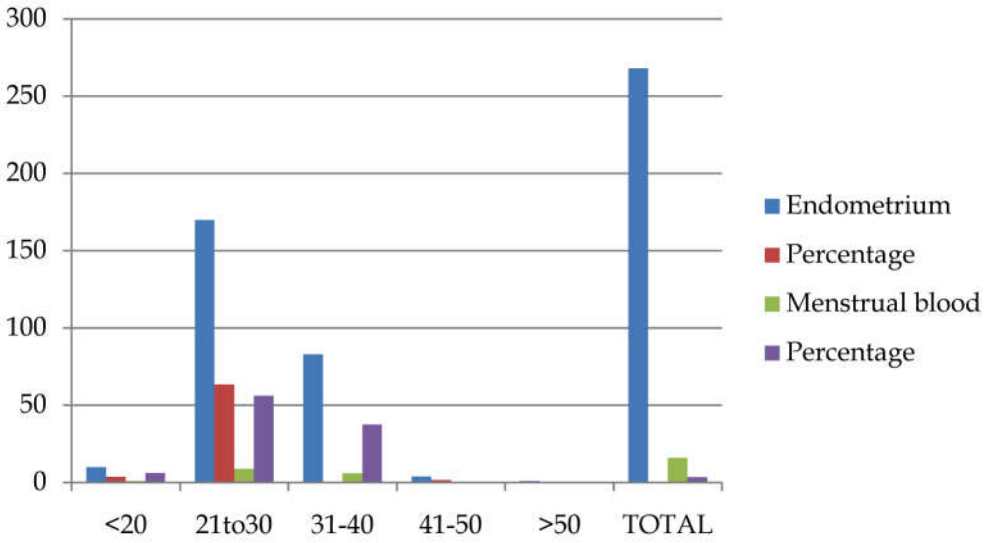
Table 5: Age wise distribution of patients(endometrium & menstrual blood)

Age	Endometrium	Percentage	Menstrual blood	Percentage
<20	10	3.73	1	6.25
21to30	170	63.4	9	56.25
31-40	83	30.90%	6	37.5
41-50	4	1.59	0	0
>50	1	0.37	0	0
TOTAL	268	59.90%	16	3.57

Graph 4:



Graph 5:



Graph 6:

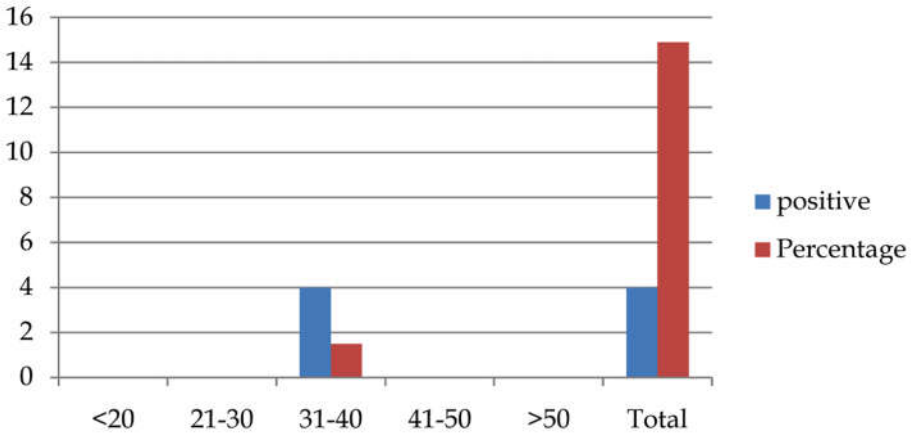


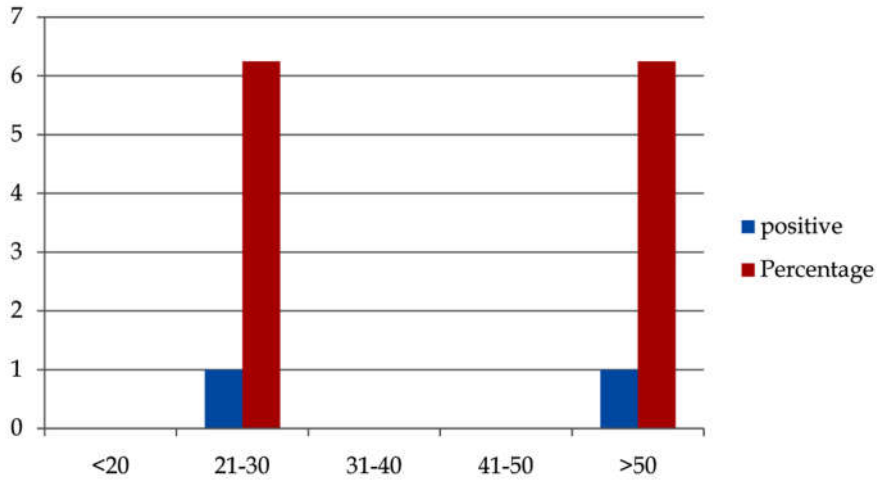
Table 6: Age wise distribution ofnumber of positive patients with endometrium

Age in years	Positive	Percentage
<20	0	0
21-30	0	0
31-40	4	1.49
41-50	0	0
>50	0	0
Total	4	14.9

Table 7: Age wise distribution ofpositive patients with menstrual blood as specimen

Age in years	Positive	Percentage
<20	0	0
21-30	1	6.25
31-40	0	0
41-50	0	0
>50	1	6.25

Graph 7:



Out of these 4.81% were detected to be positive for T.B. in 31-40 years age group and rest were negative, the overall positivity rate was 1.49% (Table 6). All positive results were correlated with histopathology of endometrium. 2 out of 4 positive cases were positive by histopathoolgy also.

The overall positivity rate with menstrual blood as sample was 6.25% (1/16) with one patient being in 21-30 years age group.Rest all were negative (Table 7).

Discussion

TB is a major health problem specially in the Indian subcontinent . The diagnosis of EPTB remains a problem because the diagnostic tools available are not sensitive enough to detect EPTB in early stages specially in paucibacillary cases.

The role of Real time PCR in diagnosis of EPTB has attracted much attention in recent years and some studies have been conducted to evaluate the role of RT PCR and the results have been variable with a sensitivity from 42 % to 100% and specificity between 85% to 100% using different PCR targets [6,7,8]. V. Mahesh Kumar et al in 2014 and M.Singh et al in 2013 observed PCR to be a sensitive and quick method to diagnose EPTB as compared to culture methods [9,10].

Sharma et al found a positivity rate of Zheil Neelson stain, culture and PCR to be 30%, 26.3% and 91.3%respectively [11]. Recently, Xpert MTB RIF assay (Cefeid Sunnyvale CA,USA) has been found to have a sensitivity of 81.2% for lymph nodes, 62.8% for CSF and 21.4% for pleural fluid [12].

Navarro Viasaro et al observed that urogenital TB was the third most frequent EPTB infection ,the first two being pleural and lymph node TB [13].

Sreerama Reddy et al observed 42.6% positive rate for lymph node TB and 14.8%in peritoneum and intestinal TB [14].

Gunal et al observed a positivity rate of 9.7% to 10.7%in TB of joints and bones ,pleura,lymph

nodes,skin and peritoneum [15].

Mazza Stalder et al in 2012 observed that the most frequent EPTB sites were lymph nodes,pleura and osteoarticular TB.They observed that peritoneum, meningeal and urogenital TB were less frequent, may be due to low sensitivity of diagnostic tests including culture and molecular amplification tests [16].

Singh et al observed a 20%-25%positive ratf EPTB with urogenital TB accounting for 4% of the burden [17].

Our findings correlate more or less with these findings in terms of frequency .But the low positivity rate (4.25%) of EPTB in our study as compared to 20% to 25% in other studies needs further analysis. It might be due to paucibacillary nature of the specimens. To overcome the limitations of our study, a larger, multicentric prospective study is required to correctly estimate the positivity rate of EPTB in Indian settings. However, the initial study on Microlab PCR system seems promising**.**

Conclusion

Truelab Micro PCR is portable,easy to use and provides accurate diagnosis of disease like EPTB with a rapid turn around time of about an hour.It is specially useful in rural areas where availability of cost effective and rapid diagnostic tools is a major problem.The diagnosis of EPTB can be made during patient's first visit only and treatment initiated as soon as the diagnosis is made.It does not require skilled manpower and elaborate infrastructure and can be easily installed in resource limited settings. A complete patient centric solution for patients suffering from EPTB is a necessity and rapid ,accurate diagnosis with Truelab MicroPCR and prompt therapywill help control TB burden in India.

Conflict of Interest

none

References

1. World Health Organization. Global Tuberculosis Control. WHO Report 2013. Geneva: WHO; 2013.
2. B. Sekar, L. Selvaraj, A. Alexis, S. Ravi, K. Arunagiri, L. Rathinavel. The utility of IS6110 sequence based polymerase chain reaction in comparison to conventional methods in the diagnosis of extra-pulmonary tuberculosis Indian J Med Microbiol, 2008; 26:352-355.
3. R. Barani, G. Sarangan, T. Antony, S. Periyasamy, A.J. Kindo, P. Srikanth. Improved detection of *Mycobacterium tuberculosis* using two independent PCR targets in a tertiary care centre in South IndiaJ Infect Dev Ctries, 2012; 6:46-52.
4. S.J. Drews, A.R. Eshaghi, D. Pyskir. The relative test performance characteristics of two commercial assays for the detection of *Mycobacterium tuberculosis* complex in paraffin-fixed human biopsy specimens Diagno Pathol, 3 (2008).
5. World Health Organization. Policy update: automated realtime nucleic acid ampli-cation technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/ RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children 2013.
6. A.K. Maurya, S. Kant, V.L. Nag, R.A.S. Kushwaha, T.N. Dhole. Detection of 123bp fragment of insertion element IS6110 *Mycobacterium tuberculosis* for diagnosis of extrapulmonary tuberculosisIndian J Med Microbiol, 2012; 30:82-186.
7. S. Chakravorty, M.K. Sen, J.S. Tyagi. Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technologyJ Clin Microbiol, 2005; 45:4357-4362.
8. S. Honoré-Bouakline, J.P. Vincensini, V. Giacuzzo, P.H. Lagrange, J.L. Herrmann. Rapid diagnosis of extrapulmonary tuberculosis by PCR: impact of sample preparation and DNA extractionJ Clin Microbiol, 2003; 41:2323-2329
9. V. Makeshkumar, R. Madhavan, S. Narayanan. Polymerase chain reaction targeting insertion sequence for the diagnosis of extrapulmonary tuber culosisIndian J Med Res, 2014; 139:161-166.
10. M. Singh,P. Singh,V.P. MyneeduEvaluation of polymerase chain reaction and Cobas TaqMan real time PCR in the diagnosis of tuberculosis: Indian prospectiveImmunol Infect Diseases, 2013; 1:1-9.
11. K. Sharma, N. Gupta, A. Sharma. Multiplex polymerase chain reaction using insertion sequence 6110 (IS6110) and mycobacterial protein fraction from BCG of Rm 0.64 in electrophoresis target genes for diagnosis of tuberculous lymphadenitisIndian J Med Microbiol, 2013; 31:24-28.
12. C.M. Denkinger, S.G. Schumacher, C.C. Boehme, N. Dendukuri, M. Pai, K.R. Steingart. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysisEur Respir J, 2014; 44:435-446.
13. Navarro-Vilasaró M., Font B., Sala M., Prera A., Malet A., Mariscal D., et al. Genitourinary mycobacteriosis: retrospective study of 45 cases in a general hospital.Enferm Infecc Microbiol Clin. 2008; 26:540–545.

14. Sreeramareddy C., Panduru K., Verma S., Joshi H., Bates M. Comparison of pulmonary and extrapulmonary tuberculosis in Nepal- a hospital-based retrospective study. BMC Infect Dis 2008; 8: 8.

15. Gunal S., Yang Z., Agarwal M., Koroglu M., Arýcý Z., Durmaz R. Demographic and microbial characteristics of extrapulmonary tuberculosis cases diagnosed in Malatya, Turkey, 2001–2007. BMC Public Health 2011; 11:154.

16. Mazza-Stalder J., Nicod L., Janssens J. Extrapulmonary tuberculosis. Rev Mal Respir 2012; 29:566–578.

17. SIngh D., Vogel M., Müller-Stöver I., El Scheich T., Winzer M., Göbels S., et al. TB or not TB? Difficulties in the diagnosis of tuberculosis in HIV-negative immigrants to Germany. Eur J Med Res 2011; 16:381–384.