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ORIGINAL ARTICLE

Rapid Diagnosis and Simultaneous Identification of Tuberculous and Bacterial Meningitis by a Newly Developed Duplex Polymerase Chain Reaction

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Abstract The present study describes the development and evaluation of a duplex polymerase chain reaction (D-PCR) for diagnosis and simultaneous identification of tuberculous meningitis (TBM) and bacterial meningitis (BM) in a single reaction. A D-PCR with primers amplifying portions of the *Mycobacterium tuberculosis* IS*6110* and the eubacteria 16SrDNA sequence in a same reaction mix was developed and tested on DNA extracted from 150 clinical CSF samples from different categories (TBM = 39, BM = 26, control infectious and non-infectious category = 85). The results indicate a clear differentiation between bands for eubacteria and *M. tuberculosis* with an analytical sensitivity of 10^3 cfu/ml for eubacteria and 10^2 cfu/ml for *M. tuberculosis*. When evaluated in clinical samples, D-PCR overall diagnosed 100 % confirmed TBM and 100 % confirmed BM cases with overall specificity of 96.5 %. D-PCR can be an effective tool for diagnosis and simultaneous identification of TBM or BM in a single PCR reaction. It saves time, cost, labour and sample amount and help in administration of appropriate antimicrobial therapy. The proposed diagnostic assay would be helpful in correct and rapid management of TBM and BM patients.

Keywords Bacterial meningitis · Tuberculous meningitis · Diagnosis · Duplex PCR

Introduction

Tuberculous meningitis (TBM) and bacterial meningitis (BM) are serious complications of central nervous system (CNS) [1] and continues to result in significant morbidity and mortality worldwide [2–5]. The aetiological diagnosis of meningitis remains a problem in clinical practice as cerebrospinal fluid (CSF) biochemical analysis findings and cellular responses often overlap for different organisms. Additional challenge encountered by clinicians in treating a case is to differentially diagnose TBM from BM or partially treated BM case. Results from biochemical, pathological analysis of CSF as well as the clinical presentation of TBM are often similar to those of BM, which results in frequent misdiagnosis and affect overall management of patient [6, 7]. Historically, a positive diagnosis of TBM and BM has required isolation of the associated organism and confirmation of its identity by performance of phenotypic tests. However, tubercle bacilli take about 6–8 weeks to grow and further in patients who have been partially treated with antibiotics before admission, the results of culture for non-TB bacterial organisms is usually negative [8]. To overcome such problems, a variety of 16S rDNA PCR assays for diagnosing BM have been developed which have consequently made a significant impact on management of meningitis [9–14]. However till date, reports of PCR assay diagnosing BM and simultaneously identifying those as TBM and BM case in one reaction are scarcely reported.

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Although the techniques are available for diagnosing both these conditions individually, detection of TBM and BM by two different PCR reactions for a single sample results in increased cost, time duration, use of labour and sample loss. The present study reports a newly developed, simple, reliable and robust duplex PCR (D-PCR) that can be used to diagnose and distinguish between a case of BM and TBM in one reaction. For this assay, we selected a broad-range primer for 16SrDNA sequence that could detect most bacterial pathogens and primer for IS*6110* region of *Mycobacterium tuberculosis*. To evaluate the test as a diagnostic tool, it was applied on DNA extracted from clinical CSF samples classified under different categories to demonstrate that its use was practical and applicable. The proposed assay aims to reduce cost, time and sample use. The usefulness of this diagnostic test would also be to eliminate unnecessary administration of antimicrobial therapy to some patients.

Materials and Methods

Control Strains

Bacterial strains used as controls in the D-PCR assay included *Haemophilus influenzae* (ATCC 19418), *Neisseria meningitidis* (ATCC 13090), *Klebsiella pneumoniae* (ATCC 10031), *M. tuberculosis* (ATCC 25177), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Streptococcus pneumoniae* (ATCC 10015), *Pseudomonas aeruginosa* (ATCC 19154) and *Acinetobacter* spp. (ATCC BAA-747).

Determination of Analytical Sensitivity

Serial tenfold dilutions of control strains of *P. aeruginosa* and *M. tuberculosis* were prepared for determination of analytical sensitivity of the assay.

Clinical Samples

CSF samples from a total of 150 patients were evaluated prospectively using the D-PCR. Patients for this study were admitted to the Neurology Department of Central India Institute of Medical Sciences, Nagpur. The Institutional Ethics Committee approved the study and the study was conducted in Central India Institute of Medical Sciences, Nagpur, Maharashtra, India. The clinical diagnosis of the patients was based on the criteria described below.

Inclusion and Exclusion Criteria

This study includes patients suspected to be infected with *M. tuberculosis* or other non-TB bacterial organisms based on their clinical characteristics, and for whom the follow-up in response to anti-tuberculosis treatment (ATT) and broad spectrum antibiotic treatment was available. Patients were excluded from TBM and BM group if there was microbiological and/or clinical evidence of another CNS infection (viral and fungal meningitis). Patients included in the study group were in between 16 and 73 years of age consisting of females and males in 1.22/1 ratio. Age and gender matched controls were taken for the control group.

Sample Size Calculation

For this study CSF samples of different groups were compared with a *Z* test and the equation for sample size is $N = 2[Zcrit\ Sqrt(2\ (p1 + p2)/2(1-(p1 + p2)/2 + Zpwr\ Sqrt(p1(1-p1) + p2(1-p2)]2/D2$, where p1 and p2 are pre study estimates of the two proportions to be considered. $D = [p1-p2]$ and Zcrit and Zpwr are defined as table value. We considered 90 % of accuracy and Significance Critiration of 0.05 and a power of 0.90. With these assumption $p1 = 0.80$, $p2 = 0.90$ $D = 0.10$, $p = 0.85$ $Zcrit = 1.960$ and $Zpwr = 0.842$. After putting all the values in formula calculated sample size is 144 patients which is statistically better to take for study.

TBM Group (n = 39)

Confirmed TBM Patients (n = 8)

Confirmed by the presence of *M. tuberculosis* in CSF by acid fast bacilli (AFB) staining and/or culture of the organism using BacT/Alert 3D (Biomeriux Inc., Durham, NC).

Clinically Suspected TBM Patients (n = 31)

TBM diagnosis was based on clinical features including sub acute or chronic fever and signs of meningeal irritation with or without other features of CNS abnormality and good response to ATT. Recruitment of patients in this group was done as per the laboratory findings reported earlier by us [15, 16].

BM Group (n = 26)

Confirmed BM Patients (n = 15)

Presence of pathogenic bacteria in CSF by gram staining and/or BacT/Alert 3D culture for bacteria other than *M. tuberculosis*.

Clinically Suspected BM Patients (n = 11)

BM diagnosis was based on clinical features including fever and/or signs of meningeal irritation or high fever and/

or signs of meningeal irritation with or without CNS manifestations and good clinical response to broad spectrum antibiotics [15, 16].

Control Group (n = 85)

Control Infectious Group (n = 28)

This group included non-TBM and non-BM infectious patients of viral and fungal meningitis with the observations as reported earlier [15, 16] along with good clinical response to antiviral and antifungal drugs respectively.

Control Non-infectious Group (n = 57)

All other patients who had no evidence of CNS or extra CNS bacterial or viral infections were included in this group. These had chronic headache, hypertension, head injury, paraparesis, dementia, myelopathy, acute cerebellitis and epilepsy.

Microbiological Investigations

CSF samples approximately 3–4 ml were initially available for the study. A total of 2 ml of CSF samples were used for routine biochemical and microbiological tests. Briefly, 2 ml of CSF was centrifuged, and a portion of the pellet was examined by Gram, India ink and Ziehl-Neelsen stains. The remaining portion of the pellet was cultured on blood and chocolate agar for bacteria and fungi and on BacT/Alert 3D, an automated microbial detection system, according to manufacturer's instruction. Two types of media bottles BacT/Alert PF and BacT/Alert MP bottles were used for culturing of BM causing organisms and *M. tuberculosis* respectively. For observing the growth of BM organisms, the pellet was added in PF bottles and incubated for 5 days and discarded on 6th day. For *M. tuberculosis* culturing, the inoculums was inoculated in MP bottles and monitored for 6 weeks or until an alarm signal indicated mycobacterial growth.

Chelex Based DNA Extraction

The DNA isolation was carried out as per the protocol previously reported by us [17]. Approximately 1–1.5 ml of sample was used to extract DNA. Cells were harvested from CSF and given 70 % ethanol treatment on ice for 20 min. This treatment completely sterilized the cultures and samples. The suspension was then centrifuged at 12,000 rpm for 5 min and the pellet was subjected to lysis with 200 µl of 20 % of a Chelex-100 suspension (pH 10.4) prepared in TEX buffer (10 mM Tris [pH 8.0], 0.5 mM EDTA and 1 % Triton X-100) with 3 µl of 10 mg/ml proteinase K. The suspension was incubated for 1 h at 55 °C to remove PCR inhibitors and was heated for 15 min at 100 °C to ensure complete cell lysis. The boiled mixture was centrifuged to pellet out the Chelex-100 resin and the supernatant was treated with ethanol for 1 h to get the precipitate. The DNA pellet recovered after centrifugation at 12,000 rpm for 10 min was subjected for drying and was dissolved in 1X TE buffer. The DNA thus isolated was stored at −20 °C and was subsequently used for PCR assays.

D-PCR

For the development of D-PCR, a eubacterial broad-range and *M. tuberculosis* primer pairs were used. The protocol took advantage of competitive DNA amplification due to which when *M. tuberculosis* was present, amplification of smaller and repetitive units of IS*6110* region was favoured in spite of presence of 16SrDNA sequence. However, when eubacteria other than *M. tuberculosis* was present amplification of only 16SrDNA occurred. Thus, the system enabled detection of either TBM or BM case in a single reaction in spite of presence of both primers.

Primers

Identification of non-TB bacterial organisms was done by using a broad range primers [18] Ul-5′-CCA GCA GCC GCG GTA ATA CG-3′, and U2-5′-ATC GG(C/T) TAC CTT GTT ACG ACT TC-3′. Identification of *M. tuberculosis* was done using a specific pair of primers designed to amplify an insertion sequence IS*6110* in the *M. tuberculosis* complex and the expected band size was about 123-bp. The sequence of IS*6110* primers, T4 and T5, were: 5′-CCT GCG AGC GTA GGC GTC GG-3′ and 5′-CTC GTC CAG CGC CGC TTC GG-3′ respectively [17].

D-PCR Assay

The optimized reagent concentrations for IS*6110* PCR and 16SrDNA PCR were combined. The protocol for D-PCR for 50 µl reaction was as follows: 0.12 µM of IS*6110* primer, 0.04 µM of each primer of 16SrDNA primer, 400 µM of dNTPs, 2.5 mM $MgCl_2$ and 0.25 U of Taq polymerase enzyme. The optimized cycling conditions were: initial denaturation at 94 °C for 5 min followed by 15 cycles of amplification each of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, followed by 25 cycles of amplification each of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. Final extension was done at 72 °C for 5 min. The preparation of master mix was carried out in a separate a PCR/UV workstation. To prevent cross-contamination, different sets of pipettes and distinct work areas

were used for DNA template preparation, PCR mixture preparation, DNA amplification and gel analysis. Moreover, two positive controls (*M. tuberculosis* and *P. aeruginosa* DNA in two separate reaction tubes) and one negative control were included with every set of samples, used during DNA extraction and D-PCR.

Interpretation of the Results by D-PCR

Agarose gel electrophoresis was used to analyze results. Interpretation of the D-PCR was considered as positive for BM when band of only 1,000-bp was present and positive for TBM when only band of 123-bp was present.

Sequencing of PCR Products

The PCR products were separated on a 2 % agarose gel, purified and sequenced by Sanger sequencing method at the SciGenom Labs, Cochin India. Sequences were verified by a BLAST search using the NCBI website.

Statistical Analysis

Sensitivity was calculated as the number of true positives/(number of true positives + number of false negatives), and the specificity was calculated as the number of true negatives/(number of true negatives + number of false positives).

Results

The analytical specificity of the D-PCR assay was assessed by applying it to DNA extracted from 8 different bacterial cultures i.e., *H. influenzae*, *N. meningitidis*, *K. pneumoniae*, *S. aureus*, *E. coli*, *S. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp. responsible for causing BM and *M. tuberculosis* responsible for TBM. All eight non-TB bacterial DNA gave amplification of only the correct predicted 16S rRNA gene PCR product size of 1,000-bp, while no Mycobacterium specific IS*6110* PCR product of size 123-bp or non-specific amplification were generated for any of them. Similarly, *M. tuberculosis* DNA upon amplification only produced band of 123-bp product and no 1,000-bp product (Supplementary material Figure S1). Sequence analysis of PCR products confirmed that the 123-bp product was identical with the *M. tuberculosis* IS*6110* region and the 1,000-bp product was specific for eubacteria.

The detection limits of the D-PCR test were determined by making culture cfu/ml dilutions of *P. aeruginosa* and *M. tuberculosis*, ranging from 10^6 to 10^1 cfu/ml and then subjecting it to the D-PCR assay. A PCR product from 10^3 cfu/ml was clearly visible for both dilution series, while additionally for *M. tuberculosis* dilution containing 10^2 cfu/ml was found to be positive [Supplementary material Figure S2 (a) and (b)].

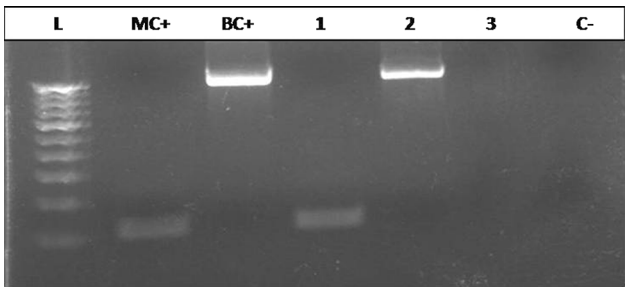

Fig. 1 Gel representing CSF sample D-PCR for amplification of IS*6110* region and 16SrDNA sequence. CSF positive samples and CSF negative samples (representing Lane number 1–3 respectively) with IS*6110* region and 16SrDNA sequence positive controls as MC+ and BC+ and negative PCR control (C−). 100-bp DNA ladder (L) was used for result analysis in agarose gel electrophoresis

The D-PCR assay was evaluated in clinical samples of suspected and confirmed TBM and BM patients (Fig. 1). The results for D-PCR on 150 clinical CSF samples are shown in Table 1. In 39 of the TBM cases (confirmed and clinically suspected) tested using the D-PCR assay, 79.5 % (31/39) were found positive. Out of 8 confirmed TBM cases by BacT/Alert 3D, D-PCR detected *M. tuberculosis* DNA in all (100 % sensitivity). In clinically suspected TBM category, D-PCR was positive in 23/31 cases (74.2 %). Out of these 23 positive cases, 15 cases were found to be positive for IS*6110* region of *M. tuberculosis* and eight were positive for 16SrDNA sequence indicating presence of eubacteria other than *M. tuberculosis* complex.

Similarly in overall BM category, 88.5 % (23/26) were found to be positive. In confirmed BM cases, D-PCR was positive in all cases (100 %). In clinically suspected category, D-PCR detected 8/11 cases (72.7 %). Out of these eight positive cases by D-PCR assay, seven were positive for 16SrDNA sequence and only one case was positive for IS*6110* region.

Regarding specificity, 3.5 % (3/85) from the overall control group (control infectious and control non-infectious group) patients were positive in the D-PCR assay thus accounting for overall specificity of 96.5 %. Out of these three positive cases, 02/28 (7.1 %) were from the other infectious category and among them, one case was found to be positive for 16SrDNA sequence and one for IS*6110* region; whereas 01/57 (1.8 %) was from the non-infectious category in the control group and was positive for 16SrDNA sequence.

Discussion

Molecular methods have already been successfully implemented for the rapid detection and differentiation of

Table 1 D-PCR results in clinically suspected TBM, PM and control group

Category (n = 150)	D-PCR positive			D-PCR negative
	Total	Only 16SrDNA	Only IS*6110*	
TBM Group = 39	31 (79.49 %)	8	23	08 (20.51 %)
Confirmed TBM = 08	08 (100 %)	0	8	0
Clinically suspected TBM = 31	23 (74.19 %)	8	15	08 (25.81 %)
BM group = 26	23 (88.46 %)	16	7	03 (11.54 %)
Confirmed BM = 15	15 (100 %)	15	0	0
Clinically suspected BM = 11	08 (72.73 %)	7	1	03 (27.27 %)
Control group = 85	03 (3.53 %)	2	1	82 (96.47 %)
Control infectious = 28	02 (7.14 %)	1	1	26 (92.9 %)
Control non-infectious group = 57	01 (1.75 %)	1	0	56 (98.25 %)

various pathogens causing meningitis. Simple PCR based methods which detects an infectious meningitis case and simultaneously distinguish it as a TBM or a BM are still scarce. These are however urgently required keeping in mind that CSF picture and clinical evidences are not able to distinguish between these two conditions which affects overall management of patient. In this study, a new D-PCR was introduced and evaluated in clinical CSF samples for their applicability to the diagnosis and simultaneous identification of TBM and BM in a single reaction.

For PCR analysis, a eubacterial broad-range and *M. tuberculosis* PCR assay based on a previously published primer pair [18, 19] was adapted to a D-PCR protocol. The protocol took advantage of competitive DNA amplification due to which when *M. tuberculosis* was present, amplification of smaller and repetitive units of IS*6110* region was favoured in spite of presence of 16SrDNA sequence. However, when eubacteria other than *M. tuberculosis* was present amplification of 16SrDNA only occurred. Thus, the system enabled detection of either TBM or BM case in a single reaction in spite of presence of both primers.

To date, only PCR assays that used 16SrDNA and IS*6110* primers individually have been reported. The clinical sensitivity of D-PCR assay was comparable to the previously published specific PCR assays in CSF. In confirmed TBM patients 100 % sensitivity was achieved. However, 15 of the 31 (48.4 %) cases of clinically suspected TBM category were positive for the IS*6110* region which is comparable to various other studies [20, 21]. All of the cases of confirmed BM category were positive by the D-PCR assay for the 16SrDNA sequence. We obtained an acceptable sensitivity of 87.5 % in clinically suspected BM category which was comparable to a study by Rantakokko et al. [22] who analyzed CSF samples by PCR technique using 16SrDNA primer and reported a sensitivity of 63.2 %. However, it was less than as in study by Saravoltz et al. [23] reported 100 % sensitivity by PCR technique using the same primer. Since, the methodologies have been known separately for eubacterial 16SrDNA as well as for mycobacterial IS*6110*, our study is first attempt of its kind to give a detailed account of a single tube PCR based detection differentiating eubacterial from mycobacterial in a single PCR run. Standardization and applicability of this PCR is novelty of work. The finding that the D-PCR made clearer the contrast of the amplified 16SrDNA and IS*6110* region in a single reaction makes it an inexpensive and time-saving method as compared to two different PCR techniques. Also, DNA amount extracted from CSF sample is limited. Here we only use it for one single reaction and difficulties for repeated extraction are avoided which adds potential to the use of D-PCR.

Additionally, D-PCR assay detected eight cases which were positive for non-TB bacterial organism, however considered as TBM cases according to CSF and clinical picture. CSF parameters similar to that of TBM do show the presence of other infecting organisms and such cases cannot be considered as false positive. Moreover, the patient did not improve on the initial ATT treatment and modification of treatment as for non-TB bacterial organism resulted in recovery. In the BM category only one was positive for *M. tuberculosis*. This ascertains the usefulness of the present diagnostic test in determining which empirical antimicrobial therapy should or should not be administered and potentially eliminates unnecessary administration of antimicrobial therapy to some patients.

The present D-PCR method is simple and applicable for any laboratory where PCR can be performed. However there are certain limitations. D-PCR had analytical sensitivity of 10^3 cfu/ml for eubacteria and 10^2 cfu/ml for *M. tuberculosis*. It was lower than a recently described multiplex PCR by Fang et al. [24] which utilizes 16SrDNA primer to detect and differentiate *M. tuberculosis*. This may be a disadvantage of the assay, especially in the clinically suspected category where we missed few cases of TBM (25.8 %) and BM (27.2 %) category. However, on the other hand, it lowers the risk of false-positive results due to

traces of contaminating eubacterial DNA which occurs frequently due to contamination of samples and PCR reagents with DNA from various sources [25] and thus we were able to achieve high specificity of 96.5 %. Secondly, we missed sequencing of eight positive cases for non-TB bacterial organisms, under TBM category which could have resulted in more accurate information about these cases. Lastly, although we have included essential positive and negative controls for deriving accurate results, incorporation of an internal control is absolute necessary and future studies will focus on improvement of these aspects.

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Conflict of interest There is no conflict of interest to declare.

References

1. Mani R, Pradhan S, Nagarathna S, Wasiulla R, Chandramuki A (2007) Bacteriological profile of community acquired acute bacterial meningitis: a ten-year retrospective study in a tertiary neurocare centre in South India. Indian J Med Microbiol 25:108–114. doi:10.4103/0255-0857.32715
2. Durand ML, Calderwood SB, Webe DJ, Miller SI, Southwick FS, Caviness VS, Swartz MN (1993) Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med 328:21–28. doi:10.1056/NEJM199301073280104
3. Aronin SI, Peduzzi P, Quagliarello VJ (1998) Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. Ann Intern Med 129:862–869. doi:10.7326/0003-4819-129-11_Part_1-199812010-00004
4. Tang LM, Chen ST, Hsu WC, Lyu RK (1999) Acute bacterial meningitis in adults: a hospital-based epidemiological study. QJM 92:719–725. doi:10.1093/qjmed/92.12.719
5. Singhi P, Bansal A, Geeta P, Singhi S (2007) Predictors of long term neurological outcome in bacterial meningitis. Indian J Pediatr 74:369–374. doi:10.1007/s12098-007-0062-6
6. Kashyap RS, Kainthla RP, Satpute RM, Agarwal NP, Chandak NH, Purohit HJ, Taori GM, Daginawala HF (2004) Differential diagnosis of tuberculous meningitis from partially-treated bacterial meningitis by cell ELISA. BMC Neurol 4:16. doi:10.1186/1471-2377-4-16
7. Christie LJ, Loeffler AM, Honarmand S, Flood JM, Baxter R, Jacobson S, Alexander R, Glaser CA (2008) Diagnostic challenges of central nervous system tuberculosis. Emerg Infect Dis 14:1473–1475. doi:10.3201/eid1409.070264
8. Hussein AS, Shafran SD (2000) Acute bacterial meningitis in adults: a 12-year review. Medicine 79:360–368. doi:10.1097/00005792-200011000-00002
9. Schuurman T, de Boer RF, Kooistra-smid AM, van Zwet AA (2004) Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting. J Clin Microbiol 42:734–740. doi:10.1128/JCM.42.2.734
10. Boden K, Sachse S, Baier M, Schmidt KH, Brodhun M, Husain R, Straube E, Isenmann S (2011) 16S rDNA-PCR and sequencing improves diagnosis of bacterial infection of the central nervous system. TOCCMJ 4:44–46. doi:10.2174/1874828701104010044
11. Sarookhani M, Ayazi P, Alizadeh S, Foroughi F, Sahmani A, Adineh M (2010) Comparison of 16S rDNA-PCR amplification and culture of cerebrospinal fluid for diagnosis of bacterial meningitis. Iran J Pediatr 20:471–475
12. Welinder-Olsson C, Dotevall L, Hogevik H, Jungnelius R, Trollfors B, Wahl M, Larsson P (2007) Comparison of broad-range bacterial PCR and culture of cerebrospinal fluid for diagnosis of community-acquired bacterial meningitis. Clin Microbiol Infect 13:879–886. doi:10.1111/j.1469-0691.2007.01756.x
13. Margall CN, Majo MM, Latorre OC, Fontanals AD, Dominguez GA, Prats PG (2002) Use of universal PCR on cerebrospinal fluid to diagnose bacterial meningitis in culture-negative patients. Eur J Clin Microbiol Infect Dis 21:67–69. doi:10.1007/s10096-001-0656-2
14. Millar BC, Xu J, Moore JE (2007) Molecular diagnostics of medically important bacterial infections. Curr Issues Mol Bio 9:21–39
15. Kashyap RS, Ramteke SS, Morey SH, Purohit HJ, Taori GM, Daginawala HF (2009) Diagnostic value of early secreted antigenic target-6 for the diagnosis of tuberculous meningitis patients. Infection 37:508–513. doi:10.1007/s15010-009-8261-x
16. Kashyap RS, Dobos KM, Belisle JT, Purohit HJ, Chandak NH, Taori GM, Daginawala HF (2005) Demonstration of components of Antigen 85 complex in cerebrospinal fluid of tuberculous meningitis patients. Clin Diagn Lab Immun 12:752–758. doi:10.1128/CDLI.12.6.752
17. Nagdev KJ, Kashyap RS, Deshpande PS, Purohit HJ, Taori GM, Daginawala HF (2010) Determination of polymerase chain reaction efficiency for diagnosis of tuberculous meningitis in Chelex-100 extracted DNA samples. Int J Tuberc Lung Dis 14:1032–1038
18. Kalghatgi AT, Praharaj AK, Sahni AK, Pradhan P, Kumaravelu S, Prasad PL, Nagendra A (2008) Detection of bacterial pathogens in cerebrospinal fluid using restriction fragment length polymorphism. MJAFI 64:29–32. doi:10.1016/S0377-1237(08)80141-4
19. Nagdev KJ, Kashyap RS, Deshpande PS, Purohit HJ, Taori GM, Daginawala HF (2010) Comparative evaluation of a PCR assay with an in-house ELISA method for diagnosis of tuberculous meningitis. Med Sci Monit 16:CR289–CR295
20. Narayanan S, Parandaman V, Narayanan PR, Venkatesan P, Girish C, Mahadevan S, Rajajee S (2001) Evaluation of PCR using TRC(4) and IS6110 primers in detection of tuberculous meningitis. J Clin Microbiol 39:2006–2008. doi:10.1128/JCM.39.5.2006
21. Michael JS, Lalitha MK, Cherian T, Mathai D, Abraham OC, Brahmadattan KN (2002) Evaluation of polymerase chain reaction for rapid diagnosis of tuberculous meningitis. Indian J Tuberc 49:133–137
22. Rantakokko-Jalava K, Nikkari S, Jalava J, Eerola E, Skurnik M, Meurman O, Ruuskanen O, Alanen A, Kotilainen E, Toivanen P, Kotilainen P (2000) Direct amplification of rRNA genes in diagnosis of bacterial infections. J Clin Microbiol 38:32–39
23. Saravolatz LD, Manzor O, VanderVelde N, Pawlak J, Belian B (2003) Broad-range bacterial polymerase chain reaction for early detection of bacterial meningitis. Clin Infect Dis 36:40–45. doi:10.1086/345438
24. Fang F, Xiang Z, Chen R (2000) Establishment of a multiplex PCR system to diagnose tuberculosis and other bacterial infections. J Tongji Med Univ 20:324–326. doi:10.1007/BF02888193
25. Döring G, Unertl K, Heininger A (2008) Validation criteria for nucleic acid amplification techniques for bacterial infections. Clin Chem Lab Med 46:909–918. doi:10.1515/CCLM.2008.152