PCR IN NEUROLOGICAL DISORDERS

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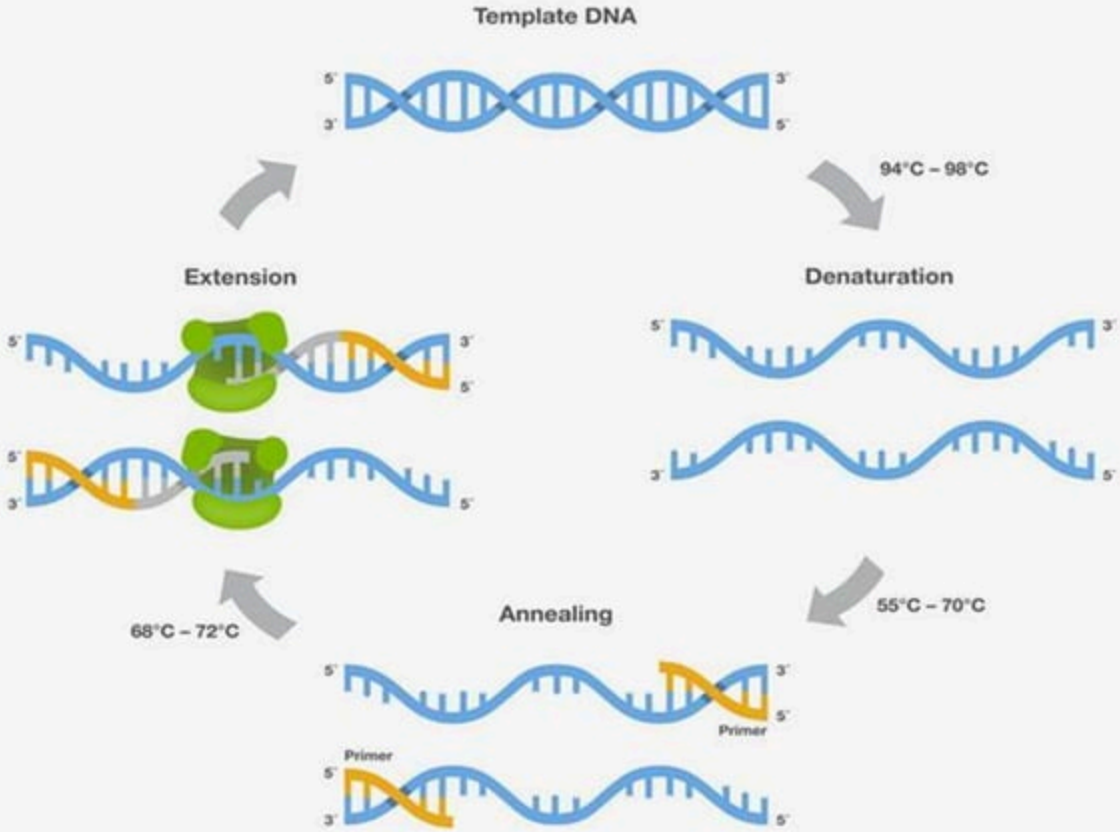
INTRODUCTION

- PCR- introduced in 1985
- Detect DNA or RNA specific to infectious organisms or a disease.
- A simple and rapid, easy-to-use approach.
- Qualitative and quantitative
- Forensic medicine ,prenatal diagnosis and molecular biology research.

-Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplificationof beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985; 230: 1350–1354.
- Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol 1987; 155: 335–350.

Method


Template DNA
5'
3'
3'
5'
94°C – 98°C
Denaturation
5'
3'
3'
5'
55°C – 70°C
Annealing
5'
3'
Primer
Primer
5'
3'
5'
68°C – 72°C
Extension
5'
3'
3'
5'
5'
3'
5'

- PCR can amplify double or single-stranded DNA
- Good for CNS infections –CSF and brain tissues sterile –monomicrobial infections-less false +ve.
- CSF lacks common inhibitors of NATs -eg, endonucleases, and exonucleases –less false neg.

- Targeted PCR-more sensitive than culture or antigen detection.
- Except for HSV and JC virus-sensitivity of most molecular tests for CNS inf. is not known.
- Multiplex or panel-based NATs .
- In 2015 FDA approved first commercial multiplex NAT for community-acquired meningitis and encephalitis .


St. pneumoniae,
N. meningitidis,
H. influenzae,
Str. Agalactiae
E. coli
L.monocytogenes
Multiplex NAT detects 14 bacterial, viral, and fungal pathogens in one hour
Enterovirus
HSV-1
HSV-2
VZV
CMV
HHV-6
hum.parechovirus
Cryptococcus neo./ gattii

PCR…

- High level of agreement with comparator methods.
- Does not detect all causes of CNS infection
- Does not provide antimicrobial susceptibility results
- Perform standard CSF bacterial and fungal cultures when ever indicated despite multiplex NAT .

FALSE NEGATIVE RESULTS

- Low sensitivity of a laboratory's assay
- Inhibition of amplification ("bloody tap," CSF fluid with high number of erythrocytes)
- Failure of the assay to amplify or detect the intended microorganism in the absence of inhibition
- Sampling error (low nucleic acid concentration)
- Failure to test for the causative organism

FALSE-POSITIVE RESULTS

- Amplification of a contaminating organism in the specimen .
- Amplification of a non-causative "bystander”
- Amplification of nucleic acid from a latent (not active) infection
- Lack of specificity of assay primer or probe sequences

CSF PCR IN SPECIFIC VIRAL INFECTIONS

Herpes simplex virus

HSE –

- Most imp. cause of sporadic fatal viral encephalitis in humans
- Effective anti-viral therapy –ACV
- Until the advent of PCR--brain biopsy(gold standard)
- HSE-direct comparison has been made between PCR and brain biopsy.

Steiner I, Budka H, Chaudhuri A, et al. Viral meningoencephalitis:a review of diagnostic methods and guidelines for management. Eur J Neurolol 2010; 17: 999-e57.

HSE…

- +Ve CSF PCR -53 of 54 =(98%) patients with biopsy-proven HSE.
- +Ve PCR -3/47 (6%) -brain tissue was culture-negative (class 1).
- Sensitivity -98%
- Specificity - 94%

Lakeman FD, Whitley RJ. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. J Infect Dis 1995; 171: 857–863.

HSE…

- CSF PCR can be recommended as a highly reliable method of diagnosing HSE without the need for brain biopsy (Level A).
- Rarely HSV-2- sp. In immunosuppressed patients and neonatal .
- CSF PCR for both HSV-1 and HSV-2.

Steiner I, Kennedy PGE, Pachner AR. The neurotropic herpes viruses: herpes simplex and Varicella-Zoster. Lancet Neurol 2007; 6: 1015–1028.

HSE…

- Timing of the CSF sample influence the sensitivity .
- CSF PCR can be negative during the early and late stages -first 3 and beyond 14 days.
- Yield is highest during the first week .
- Rx. With ACV does not reduce the chances of PCR detecting during the first week of infection (Class1)
- ACV should not influence the decision to carry out a PCR test (Level A).

-Guffond T, Dewilde A, Lobert P-E, Caparros-Lefebvre D, Hober D, Wattre P. Significance and clinical relevance of the detection of herpes simplex virus DNA by the polymerase chain reaction in cerebrospinal fluid from patients with presumed encephalitis. Clin Infect Dis 1994; 18: 744–749.
-Weil AA, Glaser CA, Amad Z, Forghani B. Patients with suspected herpes simplex encephalitis: rethinking an initial negative polymerase chain reaction result. Clin Infect Dis 2002; 34: 1154–1157.
-DeBiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. Clin Microbiol Rev 2004; 17: 903–925.

- Positive CSF PCR-most likely during day 3–14 days (class III),
- **Recommendation**- optimum timing :- 3 to 10 days after symptom onset (level C).
- If Dx. Doubtful -repeat the PCR a few days later (level B).
- Once acyclovir has been started in PCR negative susp.HSE- continue for 14 days unless an alternative diagnosis has been established (level C).

Davies NWS, Brown LJ, Gonde J, et al. Factors influencingPCR detection of viruses in cerebrospinal fluid of patients with suspected CNS infections. J Neurol Neurosurg Psychiatry 2005; 76: 82–87.

- Quantitative PCR - not a useful prognostic marker in an individual patient with HSE .
- Routine use of this technique is not recommended.

Hjalmarsson A, Granath F, Forsgren M, Brytting M, Blomqvist P, Sko¨ldenberg B. Prognostic value of intrathecal antibody production and DNA viral load in cerebrospinal fluid of patients with herpes simplex encephalitis. J Neurol 2009; 256: 1243–1251.

Varicella-Zoster virus

- VZV (chickenpox) as a primary infection - becomes latent in human ganglia.
- Decades later, it can reactivate -herpes zoster (shingles) - variety of neurological complications including post-herpetic neuralgia
- PCR has great value particularly in the absence of the characteristic rash

Steiner I, Kennedy PGE, Pachner AR. The neurotropic herpes viruses: herpes simplex and Varicella-Zoster. Lancet Neurol 2007; 6: 1015–1028.

VZV…

- PCR may reveal -VZV in cases of encephalitis of unknown cause .
- Examine the CSF by VZV PCR in such cases.
- VZV was reported to comprise as much as 29% of all confirmed or probable etiological agents in a retrospective study of 3231 cases of CNS symptoms of suspected viral origin.(Class II).
- Sensitivity and specificity - 80% and 98%, respectively .

Nagel MA, Gilden DH. The protean neurologic manifestations of Varicella-Zoster virus. Clevel Clin J Med 2007; 74: 489–504
Koskiniemi M, Rantalaiho T, Piiparinen H, et al.Infections of the central nervous system of suspectedviral origin: a collaborative study from Finland. J Neurovirol 2001; 7: 400–408.

- PCR has played a crucial role in diagnosing neurological conditions caused by VZV without rash

1. VZV vasculopathy,
2. Zoster sine herpete,
3. Myelopathy,
4. Meningoencephalitis, and
5. Polyneuritis .

Kennedy PGE. Zoster sine herpete – it would be rash to ignore it. Neurology 2011; 76: 416–417.

- Think of the diagnosis as a credible possibility.
- CSF PCR for VZV DNA should be the first investigation of choice in such cases (Level C)
-
- CSF PCR has been reported as being of lesser diagnostic value as the detection of anti-VZV IgG in the CSF (Class III)
- Appropriate Ix. are CSF and peri. blood PCR for VZV DNA and measurement of anti-VZV IgG in the CSF (Level C).

Nagel MA, Gilden DH. The protean neurologic manifestations of Varicella-Zoster virus. Clevel Clin J Med2007; 74: 489–504
Kennedy PGE. Zoster sine herpete – it would be rash to ignore it. Neurology 2011; 76: 416–417.
Gilden DH, Bennett JL, Kleinschmidt-DeMasters BK, Song DD, Yee AS, Steiner I. The value of cerebrospinal fluid antiviral antibody in the diagnosis of eurologic disease produced by varicella zoster virus. J Neurol Sci 1998; 159: 140–144.

CYTOMEGALOVIRUS

- PCR-rapid and reliable investigative tool for CNS CMV infections – sp. in AIDS patients .

1. Encephalitis and
2. Ventriculitis,
3. Acute polyradiculopathy,
4. Myeloradiculopathy,
5. Peripheral neuropathy, and
6. Retinitis

-Cinque P, Cleator GM, Weber T, et al. Diagnosis and clinical management of neurological disorders caused by cytomegalovirus in AIDS patients. European Union concerted action on virus meningitis and encephalitis. J Neurovirol 1998; 4: 120–132.
-Wolf DG, Spector SA. Diagnosis of human cytomegalovirus central nervous system disease in AIDS patients by DNA amplification from cerebrospinal fluid. J Infect Dis 1992; 166: 1412–1415.

CYTOMEGALOVIRUS

- Sensitivity = 92% and specificity = 94% were reported in one study .
- The use of CMV CSF PCR on the of patients with suspected CMV-associated neurological disease is recommended (level B).
- Quantitative CMV PCR - to determine viral load -correlate with disease severity and monitor the efficacy of anti-viral therapy .

d'Arminio Montforte A, Cinque P, Vago L, et al. A comparison of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients. J Neurol 1997; 244: 35–39.
Ginocchio CC. Laboratory diagnosis of human cytomegalovirus (HCMV) central nervous system disease in AIDS patients. Int J Antimicrob Agents 2000; 16: 447–453.

Epstein–Barr virus

- PCR has value in identifying EBV - especially in immunocompromised patents (HIV) - risk of developing primary CNS lymphoma .

1. Encephalitis
2. Aseptic neuritis,
3. Cerebellar ataxia
4. Myelitis
5. Peripheral nerve disorders -acute radicultis, radiculoplexopathy, acute autonomic neuropathy, GBS, and cranial neuropathies .

Cinque P, Brytting M, Vago L, et al. Epstein–Barrvirus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. Lancet 1993; 342: 398–401
Majid A, Galetta SL, Sweeney CJ, et al. Epstein–Barr virus myeloradiculitis and encephalomyeloradiculitis. Brain 2002; 125: 159–165.

- In AIDS patients with a suspected CNS inv., the sensitivity of CSF EBV PCR ≈ 97% in one study* and almost 100% overall ** and ≈ 98.5% specificity

- Quantitative PCR - to predict the risk of developing non-Hodgkin CNS lymphoma and for monitoring the effects of chemotherapy .

-d'Arminio Montforte A, Cinque P, Vago L, et al. A comparison of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients. J Neurol 1997; 244: 35–39. 34. Cinque P, Brytting M, Vago L, et al. Epstein–Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. Lancet 1993; 342: 398–401.
-DeBiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. Clin Microbiol Rev 2004; 17: 903–925.
-Cinque P, Bossolasco S, Lundkvist A. Molecular analysis of cerebrospinal fluid in viral diseases of the centralnervous system. J Clin Virol 2003; 26: 1–28.

Enteroviruses

- Enteroviruses (EV) - RNA viruses
- Major human pathogens
- Subgroups- poliovirus, coxsackievirus, and echoviruses .
- Children are more commonly affected
- Dis. spectrum-non-specific febrile illness, aseptic meningitis, and encephalitis and chronic meningoencephalitis(immunocompromised pts.) .

EV

- Highly effective both in terms of accurate diagnosis and improved patient management.
- RT-PCR for EV - rapid and very accurate diagnosis <24 h and much more quickly than is possible with standard viral culture (Class II).
- Sensitivity -86–90% and a specificity of 92–100%

-Rotbart HA, Sawyer MH, Fast S, et al. Diagnosis of enteroviral meningitis by using PCR with a colorimetric microwell detection assay. J Clin Microbiol 1994; 32: 2590–2592.
-Sawyer MH, Holland D, Aintablian N, Connor JD, Keyser EF, Waecker NJ Jr. Diagnosis of enteroviral central nervous system infection by polymerase chain reaction during a large community outbreak. J Pediatr Infect Dis 1994; 13: 177–182.

- PCR of specimens from the respiratory and gastro-intestinal tracts yielded higher results than did CSF.

- In any patient, child, or adult- with features of meningoencephalitis - prompt analysis of the CSF PCR for EV, is recommended (Level B).

JC Virus

- JC Virus (JCV) is a polyoma virus that is the causative agent of PML – mainly in AIDS.
- Natalizumab therapy in MS patients .
- The use of CSF PCR to detect JCV DNA has superseded brain biopsy
- sensitivity-50 –82% ,specificity 98.5–100% (Class II).

-Berger J, Concha M. Progressive multifocal leukoencephalopathy: the evolution of a disease once considered rare. J Neurovirol 1995; 1: 5–18.
-Steiner I. Quantitative risk-benefit analysis of natalizumab. Neurology 2009; 72: 1791–1792.
Weber T, Turner RW, Frye S, et al. Progressive multifocal leukoencephalopathy diagnosed by amplification of JC virus-specific DNA from cerebrospinal fluid. AIDS 1994; 8: 49–57.
-Koralnik IJ, Boden D, Mai VX, Lord CI, Letvin NL. JC virus DNA load in patients with and without progressive multifocal leukoencephalopathy. Neurology 1999; 52: 253–260.

- In a suspected case of PML, a CSF specimen should be analyzed for JCV DNA using PCR (Level B).
- In PCR negative cases-brain biopsy should be seriously considered for Def. Dx.
- CSF JC virus loads is inversely related to survival times .and lower CSF JC virus loads are predictive of longer survival times in PML [52]. JC virus loads have also been used to monitor the effects of antiviral therapy in PML patients .

García de Viedma D, Infantes MD, Miralles P, et al. JC virus load in progressive multifocal leukoencephalopathy: analysis of the correlation between the viral burden in cerebrospinal fluid, patient survival, and the volume of Neurological lesions. Clin Infect Dis 2002; 34: 1568–1575.

Human immunodeficiency virus

- Diagnosis - peripheral blood
- Quantitative PCR to assess neurological involvement in HIV infection such as HAD and encephalitis
- Can also be used to monitor therapy
- CSF viral load decreases -following HAART

-McArthur JC, McClernon DR, Cronin MF, et al. Relationship between human immunodeficiency virusassociated dementia and viral load in cerebrospinal fluid and brain. Ann Neurol 1997; 42: 689–698.
-Cinque P, Bestetti A, Morelli P, Presi S. Molecular analysis of cerebrospinal fluid: potential for the study of HIV-1 infection of the central nervous system. J Neurovirol 2000; 6: S95–S102.

Human T-cell lymphotropic virus-1

- Associated with tropical spastic paraparesis and HTLV-1-associated myelopathy.
- PCR has been shown to have a role in the diagnosis of these conditions.
- A combination of CSF PCR for proviral DNA and the antibody index for intrathecal anti-HTLV-1 antibody synthesis has been reported as providing consistent criteria for the diagnosis of these two neurological conditions
- Sensitivity and specificity of 99.4% and 98.5%, compared to western blot analysis(class III, Level C)

Scand J Infect Dis. 2008;40(10):815-20. doi: 10.1080/00365540802227102.

Usefulness of RT-PCR for the diagnosis of Japanese encephalitis in clinical samples.

Swami R[1], Ratho RK, Mishra B, Singh MP.

⊕ Author information

Abstract

The present study was carried out between July 2003 and December 2005 in PGIMER, Chandigarh, India and aimed to compare IgM capture ELISA and nested RT-PCR for the diagnosis of Japanese encephalitis (JE). The samples collected were cerebrospinal fluid and blood from 40 febrile patients with encephalitis (n=40, group I) and blood samples from febrile patients without encephalitis residing in JE endemic areas (n=45, group II). Overall, in CSF samples JE specific RNA was detected in 9/40 (22.5%), while 7/28 (25%) patients showed the presence of specific IgM antibodies. Only 28 CSF samples could be subjected to both RT-PCR and IgM and, among these, 13 cases were found to be confirmed JE based on IgM and/or RT-PCR positivity. Among the confirmed cases, 6 (6/13, 46.5%) could be detected by RT-PCR alone, 4 (4/13, 30.7%) by IgM capture ELISA and 3 (3/13, 23.1%) patients were positive by both the methods. All the RT-PCR positive cases had presented within 5 d of onset of illness. The serum samples of only 16 patients in group I could be tested for IgM antibodies and 5 (31.25%) were found to be positive, while in group II, 11.1% (5/45) positivity was observed. JE specific RNA could not be detected in serum samples of either group of patients. This study highlights the need for carrying out RT-PCR in CSF samples, compared to IgM antibody detection, for the early detection of JEV.

Table 1 Recommendations for the use of PCR for the diagnosis of CNS viral infections

Virus	Reported sensitivity and specificity of CSF PCR	Evidence class and level of recommendation
Herpes simplex virus (HSV)-1	96% and 99% [15]	Class 1 Level A
Encephalitis		May be false negatives during first 3 days
Varicella-Zoster virus (VZV)	80% and 98% [25]	Class III Level C
		CSF anti-VZV IgG more sensitive than PCR in VZV vasculopathy
Cytomegalovirus (CMV)	92% and 94% [32]	Class II Level B
		Quantitative PCR may also be clinically useful
Epstein–Barr Virus (EBV)	97–100% and 98.5% [33,34,36]	Class IV Level C
		Quantitative PCR may also be clinically useful
Enteroviruses	31–95% and 92–100% [37,40, 41]	Class II Level B
JC virus (JCV)	50–82% and 98.5–100% [48–50]	Class II Level B
		Quantitative PCR may also be clinically useful
Human immunodeficiency virus (HIV)	Diagnosis will already have been made on the blood	CSF viral load a useful tool in assessing neurological involvement
Human T-cell lymphotropic Virus (HTLV-1)	75–99.4% and 98.5% [40,57]	Class III Level C
		Combination of CSF PCR and anti-HTLV-1 antibody index
		useful in diagnosis

Bacteria

Bacteria

- PCR- available for the past two decades.
- Conventional diagnostic methods are time consuming ,may be negative or non-diagnostic.
- Prior empirical AB Rx –Neg. microscopy and culture
- CSF-immunology may not always distinguish between an active infection and previous exposure or partially treated remote infection.

- PCR results - within 24–36 hr. [may be as low as1.5 hr.] and utilize low volume of CSF (1 ml)

- Real-time quantitative multiplex PCR -highly sensitive technique - can detect as few as

1. 2 copies of N. meningitidis, Strep.pneumoniae, and E. coli,
2. 16 copies of Listeria monocytogenes,
3. 28 copies of group B streptococcus

Chiba N, Murayama SY, Morozumi M, et al. Rapid detection of eight causative pathogens for the diagnosis of bacterial meningitis by real-time PCR. J Infect Chemother 2009; 15: 92–98.

Acute meningitis

- Currently available PCR methods detect Hemophilus influenzae, N. meningitidis, S. pneumoniae, L. monocytogenes in CSF
- Sensitivity of 87– 100% and specificity of 98–100%
- Presently, quantitative multiplex RT-PCR appears to be the preferred technology for acute bacterial meningitis
- Negative bacterial PCR assay virtually excludes the diagnosis

Saravolatz LD, Manzor O, VanderVelde N, Pawlak J, Belian B. Broad range bacterial polymerase chain reaction for early detection of bacterial meningitis. Clin Infect Dis 2003; 36: 40–45.

Chronic meningitis

- TBM remains a major challenge, both in adults and in children
- Low sensitivity of smear positivity (<10%)
- Prolonged period of culture time
- Quantitative RT-PCR - increase diagnostic yield in TBM.
- Sensitivity of PCR-based tests in CSF - 46% to 66% and specificity from 97% to 99% .

TBM

- A novel RT-PCR-based rapid detection method (Xpert MTB/RIF assay)- easy to use and shown great promise in identifying drug-resistant .

- Its value in the detection of drug-resistant mycobacterial strains in CSF samples is yet to be tested.

Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampicin resistance by use of on-demand, near patient technology. J Clin Microbiol 2010; 48: 229–237

TBM

- PCR-based methods are as prone as conventional cultures for cross-contamination
- Diagnostic specificity may be compromised in endemic areas .
- May be falsely positive

Thwaites GE, Caws M, Chau TT, et al. Comparison of conventional bacteriology with nucleic acid amplification amplified mycobacterium direct test) for diagnosis of tuberculous meningitis before and after inception of antituberculous chemotherapy. *J Clin Microbiol* 2004; 42: 996–1002.

- Negative CSF PCR result does not exclude the diagnosis of neurotuberculosis in an appropriate clinical setting when supported by the CSF and imaging data.

- Mycobacterial DNA may persist for up to a month in CSF after starting therapy

- Repeat quantitative rt-pcr test may aid to diagnosis even if initial PCR result is negative

Int J Tuberc Lung Dis. 2016 May;20(5):625-30. doi: 10.5588/ijtld.15.0741.

Multitargeted loop-mediated isothermal amplification for rapid diagnosis of tuberculous meningitis.

Modi M[1], Sharma K[2], Sharma M[2], Sharma A[3], Sharma N[3], Sharma S[1], Ray P[2], Varma S[3].

⊕ Author information

Abstract

SETTING: Rapid and accurate diagnosis of tuberculous meningitis (TBM) is imperative for the optimal management of patients. Loop-mediated isothermal amplification (LAMP) is a promising nucleic-acid amplification assay, especially for resource-poor, endemic countries.

OBJECTIVE: Evaluating LAMP assay using insertion sequence (IS) 6110 and MPB64 targets for the Mycobacterium tuberculosis complex (MTC) for the rapid diagnosis of TBM. Results were compared with culture and the composite reference standard.

DESIGN: The LAMP assay was performed using six MTC-specific primers each for IS6110 and MPB64 on the cerebrospinal fluid of 150 TBM patients (50 confirmed, 100 suspected) and 100 non-TBM control subjects.

RESULTS: Multitargeted LAMP had a sensitivity and specificity of 96% and 100% for confirmed (50 culture-positive) TBM cases. The sensitivity of IS6110 polymerase chain reaction (PCR), IS6110 LAMP and MPB64 LAMP for probable cases was respectively 70 (70%), 78 (78%) and 82 (82%). In a total of 150 TBM patients, the overall sensitivity of microscopy, IS6110 PCR, IS6110 LAMP, MPB64 LAMP and the multitargeted LAMP was respectively 4%, 74.6%, 82.7%, 86.7% and 88%. The specificity for all was 100%. Six cases were missed by IS6110 LAMP and two cases by MPB64 LAMP.

CONCLUSION: The LAMP assay using two targets is a promising and accurate technique for the rapid diagnosis of TBM.

PMID: 27084816 DOI: 10.5588/ijtld.15.0741

[Indexed for MEDLINE]

Recommendation

- The diagnostic yield -influenced by the time to test after initiation of antibiotic therapy.
- Repeating CSF PCR within first 3 weeks may aid diagnosis in tuberculous meningitis if the initial result is negative (Class IV, Grade C).
- CSF- PCR is not presently a validated diagnostic test for Lyme neuroborreliosis (Class IV, Grade C)

- In cases of suspected meningococcal meningitis, an early microbiological diagnosis is necessary - to prevent secondary cases.
- However, PCR based diagnostic tools should be used as an adjunct

Recommendations

- Quantitative RT-PCR is a valuable adjunct for diagnosis of bacterial meningitis and is recommended for routinevbuse in CSF samples (class II, grade A)

- Direct microscopy and culture remain the gold standard of bacterial infections of central nervous system where feasible and current bacterial PCR tests do not replace them (class II grade A).

Parasites

- PCR can directly detect parasite DNA/RNA - highly sensitive and specific, is still too expensive.
- In immuno-compromised patients, indirect diagnostic methods (serology for antibody detection, etc.) have a very low sensitivity.
- PCR is essential for earliest possible diagnosis and management.

Parasites…

- diagnosis -rests upon clinical signs and symptoms, clinical history, travel history including geographic exposure and, finally, on laboratory techniques

- The primary tests-particularly in tropical, resource-poor areas, have not changed during the past decades.

Parasites…

- Light microscopy still being the diagnostic mainstay.

- Indirect methods, (serology), can not distinguish between past, latent, reactivated, or acute infection and are of little use in ascertaining therapy response or for prognosis.

Muldrew KL. Molecular diagnostics of infectious diseases. Curr Opin Pediatr 2009; 21: 102–111.

Parasites…

- PCR and RT-PCR have been introduced only in cerebral toxoplasmosis for routine diagnosis in immuno-compromised patients.
- The other molecular-based assays have not yet replaced serology or light microscopy.
- they cannot yet replace fully (with the exception of toxoplasmosis) the light microscopy or serology

- in helminthic diseases, the direct visualization or detection of the helminths still represents the gold standard.

Recommendations

- Microscopy and serology show many limitations .
- PCR can detect infestations or infections from samples with very low burden of parasites
- Higher sensitivity and enhanced specificity compared with existing diagnostic tests.
- Used as the reference diagnostic tool in european laboratories and research purposes in tropical areas.

Table 2 Use of PCR for the diagnosis of CNS protozoonoses

Protozoal pathogen	CNS manifestation	Molecular-based diagnostic technique	Evidence class	Recommendation
Free living amebae	Granulomatous amebic encephalitis	PCR [85]	IV	–
		Nested PCR [86]		
Acanthamoeba spp.	Acute primary amebic meningoencephalitis	Real-time PCR [87]		
Balamuthia mandrillaris		PCR [88]		
Naegleria fowleri		Real-time PCR [89]		
		Multiplex real-time PCR [90]		
Entamoeba histolytica	Brain abscess	Real-time PCR [91]	In stools	–
		Multiplex tandem real-time PCR [92,93]	II	
			In abscess aspirate	
		High through put multiplex PCR [84]	IV	
		Probe-based detection with luminex beads [84]		
Babesia microti	Anemia, hypoxic encephalopathy	PCR [94,95]	IV	–
Plasmodium falciparum	Cerebral malaria, multi-organ malaria	Real-time PCR [96]	II (depending whether patient lives in holoendemic region or in non-endemic region)	C
		PCR multiplex real-time PCR [80,97] quantitative nucleic acid sequence-based amplification [98]		
		Real-time quantitative nucleic acid sequence-based amplification [99]		
		Loop-mediated isothermal amplification [100–102]Polymerase chain reaction,ligase detection reaction fluorescent microsphere-based assay [103]		
		PCR ELISA [104,105]		
		Nested PCR [106]		
		Reverse transcription loop- mediated isothermal amplification (RT-LAMP) [106]		
Plasmodium knowlesi	Usually severe anemia Rarely: cerebral malaria	Nested PCR [107]	IV	–
Toxoplasma gondii	Cerebral toxoplasmosis (granulomata, acute encephalitis; very rare in immuno-competent, usually in immuno-compromised patients) Congenital toxoplasmosis	Quantitative polymerase chain reaction [108]	I	B
		Rapid-PCR (B1-gene) [109,110]		
		Loop-mediated isothermal amplification [111]		
Trypanosoma cruzi	Acute meningoencephalitis, myocarditis, in chronic Chagas disease: cardio-embolic stroke	PCR [112–114]	II	B
		Loop-mediated isothermal amplification [114]		
Trypanosoma brucei gambiense and *Trypanosoma brucei rhodesiense*	Chronic (*T. b. gambiense*) or sub-acute (*T. b. rhodesiense*) meningoencephalitis Sleeping sickness	PCR [117–119]	II	B
		Real-time PCR [120]		
		Nucleic acid sequence-based amplification and PCR coupled to oligo-chromatography [121]		
		Loop-mediated isothermal amplification (LAMP) [122]		

Table 3 Use of PCR for the diagnosis of CNS helminthoses

Helminth	CNS manifestation	Molecular-based diagnostic technique	Evidence class	Recommendation
Angiostrongylus cantonensis	Eosinophilic meningitis	Multiplex PCR [123] Loop-mediated isothermal amplification assay [124]	IV	
Echinococcus granulosus	Cystic echinococcosis (space-occupying intracranial cyst)	Direct-PCR [125]	IV	
Filarial species *Wuchereria brancofti*, *Brugia malayi*	Lymphatic filariosis, rarely: neurofilariasis (cerebral larva migrans)	Real-time PCR [126]	IV	
Paragonimus westermani	Space-occupying intracranial cyst	Multiplex PCR [127] Loop-mediated isothermal amplification [128]	III	
Schistosoma spp.	Space-occupying granuloma intracerebral and spinal space- occupying granuloma	PCR [129,130] Real-time PCR [131,132] Polymerase chain reaction – oligochromatic dipstick [133]	II	
Strongyloides stercoralis	*Strongyloides stercoralis* hyperinfection syndrome (in the immune-compromised) with fulminant meningitis and sepsis syndrome (accompanying gram negatives)	PCR [134] Real-time PCR [135] Pentaplex-real-time PCR [136] High throughput multiplex PCR and probe-based detection with luminex beads [87] Duplex-real-time PCR [137]	II	B
Taenia solium – larval stage: *Cysticercus cellulosae*	Neurocysti-cercosis (Space-occupying, cystic intracranial lesions, epilepsy)	PCR [138,139] Nested PCR [140] Semi-nested PCR [141] PCR amplified DNA sequences targeting *T. solium* mitochondrial cox1 gene and cob gene [142] Loop-mediated isothermal amplification [143]	II	B
Toxocara canis (cati)	Larva migrans visceralis (cerebralis, intracranial granuloma, vasculitis)	PCR [144]	IV	

FUNGAL INFECTIONS

Fungal infections

- CNS infection can be the consequence of disseminated infection or CNS confined.
- The incidence ↑ng immunocompromized individuals.
- Positive cultures together with microscopy, antigen/antibody testing in serum, and CSF are the diagnostic ‘gold standard’.

Major Drawbacks

- Slow growth
- cross-reactivity in case of Ag detection
- inadequate immune response are

Histoplasmosis (Histoplasma capsulatum)

- most common endemic mycosis in Europe
- Fungal culture is the gold standard
- antigen and antibodies can be determined in CSF for diagnosis.
- A limited number of studies evaluated the value of PCR in CNS histoplasmosis (class IV)
- no commercial kit available for routine use.

Ashbee HR, Evans EG, Viviani MA, et al. Histoplasmosis in Europe: report on an epidemiological survey from the European Confederation of Medical Mycology Working Group. Med Mycol 2008; 46: 57–65.

Cryptococcosis

- Chronic basal meningitis is the most frequent CNS manifestation
- Culture alone is generally not the method of choice.
- The diagnostic mainstay is antigen detection with >90% sensitivity and specificity .
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- India ink stain is diagnostic in 80% of AIDS and 50% in immunocompetant pt.
- There are five studies using PCR for the diagnosis of CNS cryptococcosis, three with evidence class III and two evidence class IV.
- PCR and EIA had a sensitivity and specificity of 100%. A 100% sensitivity was

Table 4 The value of PCR in the diagnosis of fungal CNS infections

	Recommendation	Reference	Country (year of publication)	Evidence class	No. of samples or patients (controls)	Specimen	Positive culture	Positive microscopy Histology/ staining/smear	Positive antigen	Positive antibody	+PCR
Histoplasmosis	–	150	Spain (2010)	IV	1	CSF					1
Coccidioidomycosis	–	154	USA (2010)	IV	5	CSF	1			2	0
		182	USA (2011)	IV	2	CSF	1			1	2
Cryptococcosis	Level C*	155	India (2009)	III	46 (30)	CSF	44	43	46		46 (0 in controls)
		156	Italy (1998)	III	21 (19)	CSF	21	21			21 (0 in controls)
		157	Brazil (2004)	III	56 (16)	CSF	43	48			52 (0 in controls)
		158	India (2002)	IV	25	CSF	25				25
		159	India (2007)	IV	17	CSF	13	13			13
Candidiasis	–	163	Sweden (2006)	IV	24	CSF		1			4
		164	Canada (2001)	IV	4	CSF	0		2		2
		165	Canada (1996)	IV	7	CSF	1				3
Aspergillosis	Level C*	172	Japan (1999)	III	5 (11)	CSF	0		EIA 4/LA 4		5 (0 in controls)
		166	The Netherlands (1999)	III	26 (30)	CSF			26	0	4 (0 in controls)
		170	Japan (1999)	IV	1	CSF	0				0
		174	Japan (2004)	IV	1	CSF			0	0	1
		175	Germany (2006)	IV	35	CSF					14
		173	The Netherlands (1999)	IV	2	CSF			1		1
		176	Italy (2002)	IV	2	CSF	2		1		1

(continued)

Recommendation

- CSF PCR for the diagnosis of suspected CNS Cryptococcosis and CNS Aspergillosis in addition to the routine methods is likely to be of value (level C recommendations).
- There are class IV evidence for CSF CNS Histoplasmosis, Coccidioides, and Candida and CNS mucormycosis.
- There is no enough evidence to recommend the use of PCR as a routine diagnostic tool in these cases.

Costs of commonly used PCR in India

- HSV-1 = 3700
- HSV-2 = 3700
- Interovirus PCR = 4000
- CMV Quanti. = 8600
- CMV Quali. = 3900
- TB PCR = 2000
- Meningitis panel [HI/NM/SP] = 4700
- Cryptococcal Neo. PCR = 9500

Other uses

- **Down syndrome-** A duplex real-time PCR assay, -DSCR4 gene on chromosome 21-rapid prenatal diagnosis of Down syndrome from amniotic fluid samples (Zhu et al. 2009).

- 100% sensitivity and 99.7% speci ficity (Ehrich et al. 2011)

- **DMD/BMD**- Multiplex PCR represents a sensitive and accurate method for deletion detection of most of the cases of DMD and BMD.

Spinal Muscular Atrophy-

- Molecular diagnosis of spinal muscular atrophy is based on use of PCR methods for detection of mutations in survival motor neuron 1 (SMN1) gene, .

Triple Repeat Disorders-

- based on PCR amplification of the triple repeat in genomic DNA obtained from peripheral blood nucleated cells.

- **CMT**

Conclusion

- Ability to provide diagnosis by PCR varies according to the group of pathogens
- Viruses >bacterial >protozoal infections and helminthic infestations >fungal infections
- More clinical research is required to test and eventually confirm its role in this group of infections.

Thank you

References

- Uptodate.com;Molecular diagnosis of central nervous system infections;Authors: Cathy A Petti et.al;Dec 02, 2015
- EFNS-ENS guidelines for the use of PCR technology for the diagnosis of infections of the nervous system I. Steinera et. al.; European Journal of Neurology 2012