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EFNS-ENS GUIDELINES/CME ARTICLE

# EFNS-ENS guidelines for the use of PCR technology for the diagnosis of infections of the nervous system

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**Background:** Polymerase chain reaction (PCR) as a means to amplify nucleic acids has become an essential element in diagnosis of infections. It has evolved into a simple and rapid, easy- to- use approach. At present there are no published guidelines for the usage of PCR technology for the diagnosis of infections of the nervous system.

**Methods:** We reviewed the advantages and pitfalls of PCR in order to guide neurologists and infectious diseases experts in its application for the diagnosis of infections of the nervous system. Medical reference systems were searched, and original papers, meta-analyses, review papers, book chapters and guidelines recommendations were reviewed. The final literature search was performed in May 2012. Recommendations were reached by consensus.

**Recommendations:** The reliability of PCR technology for the diagnosis of neurological infections is currently based on the pathogens. The main contribution of PCR is to the diagnosis of viral infections followed by bacterial CNS infections with the notable exception of tuberculous meningitis. Efficacy for the diagnosis of protozoal infections and helminthic infestations has also been established in many instances. Unfortunately, current molecular PCR technology is far from becoming routine in resource-poor countries where such infections are prevalent. Despite the importance of fungal infections in the context of the immune-compromised host, there is not enough data to recommend the routine use of PCR.

**Conclusions:** PCR technology is currently a reliable method for the diagnosis of viral and bacterial (except tuberculosis) infections, and only for some protozoal infections and helminthic infestations.

## Introduction

The introduction of polymerase chain reaction (PCR) technology in 1985 [1,2] as a means to amplify nucleic acids has revolutionized clinical medicine. It has become an essential element in diagnosis of infections and malignant conditions and is an important tool in both forensic medicine and prenatal diagnosis. It is also essential for molecular biology research. From a difficult and time-consuming technology that relied on the visual interpretation of stained gels to detect the presence of amplification products, it has evolved into a simple and rapid, easy-to-use approach. It also provides the means to perform quantitative PCR, which uses precision optics and DNA-binding fluorescent dyes or fluorescent labels to monitor amplification in real-time.

The PCR, which was developed by scientists at Cetus, involves the *in vitro* enzymatic synthesis of millions of copies of a specific DNA segment. The reaction is based on the annealing and extension of two oligonucleotide primers that flank the target region in duplex DNA; after denaturation of the DNA, each primer hybridizes to one of the two separated strands such that extension from each 3′ hydroxyl end is directed toward the other. The annealed primers are then extended on the template strand with a DNA polymerase. These three steps (denaturation, primer

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binding, and DNA synthesis) represent a single PCR cycle. If the newly synthesized strand extends to or beyond the region complementary to the other primer, it can serve as a primer binding site and template for a subsequent primer extension reactions. Consequently, repeated cycles of denaturation, primer annealing, and primer extension result in the exponential accumulation of a discrete fragment whose termini are defined by the 5′ ends of the primers. The length of the products generated during the PCR is equal to the sum of the lengths of the two primers plus the distance in the target DNA between the primers. PCR can amplify double or single-stranded DNA, and with the reverse transcription of RNA into a cDNA copy, RNA can also serve as a target.

At present, there are no published guidelines for the usage of PCR technology for the diagnosis of infections of the nervous system. The aim of the present primer is to guide neurologists and infectious diseases experts in the application of this technology to the diagnosis of infections of the nervous system.

Classification of evidence levels used in these guidelines for therapeutic interventions and diagnostic measures was according to Brainin *et al*. [3].

## Methods

We searched MEDLINE (National Library of Medicine) for relevant literature from 1966 to July 2011. The search included reports of research in human beings only and in English. The Cochrane library and the guideline section of the American Academy of Neurology were assessed on July 15th, 2011. Review articles and book chapters were also included if they were considered to provide comprehensive reviews of the topic. The final choice of literature and the references included was based on our judgment of their relevance to this subject. Recommendations were reached by consensus of all Task Force participants and were also based on our own awareness and clinical experience. Where there was lack of evidence but consensus was clear, we have stated our opinion as good practice points.

## Viruses

While not a technique optimized for all viruses, PCR has, nevertheless, provided a rapidly effective diagnostic technique that has superseded the more classical methods of tissue biopsy and serial serology using intrathecal antibody responses.

Several general points should be made prior to making specific recommendations. First, PCR not only allows a specific diagnosis in an individual patient but can also define the spectrum of disease caused by a particular virus, often by retrospective analysis, such as milder, atypical herpes simplex encephalitis (HSE) [4]. Second, PCR applied to large numbers of clinical samples can help define the range of, and frequency with which viruses cause particular neurological infections. For example, of 3231 patients with encephalitis, meningitis, or myelitis, it was found that 46% were because of a virus, with the prominent role of Varicella-Zoster virus (VZV), Herpes simplex virus (HSV), enteroviruses, and influenza A viruses being positively identified [5]. In another retrospective study of 787 CSF samples, the most frequently detected viruses were HSV, enteroviruses, and Epstein–Barr virus (EBV) [6]. In this study, it was found that the PCR examination was positive in only 5% of clinical episodes judged unlikely to be due to a CNS viral infection. Third, the advent of PCR has increased the frequency of a definite viral diagnosis, with one study of consecutive, but not blinded, samples showing that a patient with a positive PCR is 88 times as likely to have a definite diagnosis of a viral infection of the CNS compared with one in whom the PCR is negative [7] (Class III). Fourth, both the timing of the CSF sample and the physical conditions such as specimen storage can be important confounding variables in PCR diagnosis, so close attention needs to be given to these [8]. Related to this, a self-evident pre-requisite for obtaining a very high specificity for viral PCR is that the laboratory carrying out the assay is fully experienced in this technique; in particular, it is vital to avoid contamination. Fifth, more recently several viruses can be looked for in the same CSF or other sample using the technique of multiplex PCR in which several pairs of primers specific for particular viral sequences are used [9]. In such cases where more than one virus is detected in a CSF sample, the significance of the virus detected has to be carefully evaluated, especially if such a dual viral infection is made more likely by immunosuppression as occurs during Human Immunodeficiency virus (HIV) infection [10]. Sixth, vital factors in evaluating PCR results in patients' CSF are the sensitivity and specificity of the particular assay, denoting the possibility of false negative and false positive results, respectively (see below) [9]. Both can vary considerably, ranging from very high to lower values, and therefore, less satisfactory, sensitivities, and specificities. Where a direct comparison has been made between PCR results in patients' CSF and a definite tissue diagnosis, it is possible to obtain highly reliable sensitivities and specificities of over 96%, as is the case in HSE [11,12]. Seventh, in some cases, the technique of quantitative PCR has been applied in

 

which real-time PCR allows the determination of the viral load in a patients' blood or CSF [9]. Although this is not carried out routinely in most laboratories, it has been used in some cases to assess the severity of the viral disease burden and/or the prognosis, examples being Cytomegalovirus (CMV) and JC virus infections [8]. Eighth, although viral PCR in the context of the present discussion is performed primarily in neurological patients' CSF, it can also, under appropriate circumstances, be carried out on other tissues such as peripheral blood, brain, or other tissue biopsy specimens.

### Examples of the use of CSF PCR in specific viral infections of the CNS

*Herpes simplex virus*

We focus here on HSE because (i) this is the most important cause of sporadic fatal viral encephalitis in humans, (ii) there is effective anti-viral therapy for HSE with acyclovir [13] (Class 1), and (iii) the utility of CSF PCR in this condition has been investigated in many studies. Until the advent of PCR, a diagnosis of HSE could only be made definitively by brain biopsy, which is the gold standard for this condition [13]. Herpes simplex encephalitis is one of the very few neurological infections in which a direct comparison has been made between the frequency of detection of CSF PCR and brain biopsy. Thus, HSV DNA was detected by PCR in the CSF of 53 (98%) of 54 patients with biopsy-proven HSE, and a positive PCR was also detected in 3/47 (6%) of patients whose brain tissue was culture-negative [14] (Class 1). Overall, the sensitivity of CSF PCR, that is, the percentage of actual cases expected to be detected by this method was 98%, indicating a false negative rate of 2%. The specificity of CSF PCR, that is, the percentage of cases identified as being not-HSE which were in fact another condition, was 94% indicating a false positive rate of 6%. These figures have largely been confirmed in subsequent studies, and a detailed study of this issue using a decision model analysis estimated that the sensitivity of CSF PCR in HSE is 96% and the specificity is 99% [15]. Based on such data, CSF PCR can be recommended as a highly reliable method of diagnosing HSE without the need for brain biopsy (Level A). Because of the rarer association of HSV-2 with mild or atypical of cases of HSE, and in particular in immunosuppressed patients such as those with HIV infection [4], as well as neonatal HSE caused by HSV-2 [16], it is important to carry out CSF PCR for both HSV-1 and HSV-2.

The timing of the CSF sample used for PCR is an important factor influencing the sensitivity of the method. The CSF PCR for HSE can be negative during the early stage of the infection, with documented failures to detect HSV-1 DNA during the first 3 days after the onset of the illness [17,18]. Re-examination of the same negative CSF a few days later may yield a positive PCR result [18]. The CSF PCR may also be negative if the sample is taken too late during the infection [8], and this is because the yield of virus is highest during the first week of the infection following which it falls [14]. However, treatment with acyclovir does not reduce the chances of PCR detecting HSV during the first week of infection [14] (Class 1) so such treatment should not influence the decision to carry out a PCR test (Level A). A retrospective study of 787 samples for the detection of all viruses suggested that a positive CSF PCR was most likely if the samples were taken 3–14 days after the onset of symptoms [6] (Class III), and similar figures probably also pertain in the specific case of HSE where we recommend the optimum timing of CSF PCR at 2–3 days to 10 days after symptom onset (Level C). Therefore, caution should be exercised in the case of a negative CSF PCR within the first 72 h of the onset of symptoms in a case of suspected HSE. If doubt about the diagnosis continues, it seems prudent to repeat the PCR a few days later to obtain a definitive diagnosis (Level B). Once acyclovir has been started in a case of suspected HSE with a negative PCR, we recommend that it is continued for 14 days unless an alternative diagnosis has been established (Level C). The issue as to whether it is justified to repeat the CSF PCR in all patients after 14 days of acyclovir treatment as previously suggested [19] is controversial, and we do not recommend this at the current time (Level C). However, the results of the ongoing US NIH anti-viral study group trial should resolve this issue definitely.

Quantitative PCR has been used in some laboratories in cases of HSE in both adults and neonates [8,20], but a recent large retrospective study on stored frozen samples found that viral load assessed by quantitative PCR was not a useful prognostic marker in an individual patient with HSE [21]. Therefore, we do not recommend the routine use of this technique in the management of HSE.

*Varicella-Zoster virus*

Varicella-Zoster virus causes varicella (chickenpox) as a primary infection following which the virus becomes latent in human ganglia. Decades later, it can reactivate to cause herpes zoster (shingles) that may be followed by a variety of neurological complications including post-herpetic neuralgia [16,22]. PCR has been proven of great value in defining and extending

the range of neurological conditions caused by VZV reactivation, particularly when they occur in the absence of the characteristic rash [23]. PCR may reveal the etiological role of VZV in cases of encephalitis of unknown cause [24], so it is always advisable to 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 [5].(Class II). The sensitivity and specificity of VZV PCR in cases of VZV-associated neurological disease has been estimated at 80% and 98%, respectively [25]. More recently, CSF VZV viral loads in patients known to be CSF VZV-positive by PCR were measured using real-time PCR [26]. The CSF viral load was found to be significantly higher in patients with encephalitis and acute aseptic meningitis compared with patients with cranial nerve involvement.

It has become increasingly apparent that the spectrum of neurological conditions caused by VZV without reactivation is much wider than previously thought, and PCR has played a crucial role in proving this [27]. Conditions that can be diagnosed in this way include VZV vasculopathy, zoster sine herpete, myelopathy, meningoencephalitis, and polyneuritis cranialis [27]. The important aspect of management is to think of the diagnosis as a credible possibility. While CSF PCR for VZV DNA should be the first investigation of choice in such cases (Level C), this may need to be supplemented by other data. For instance, while CSF PCR is used to diagnose CNS VZV infections, this test has been reported as being of lesser diagnostic value as the detection of anti-VZV IgG in the CSF [24,28] (Class III) so both need to be carried out. In a patient suspected of having persistent radicular pain, or other neuroinflammatory condition of the brain or spinal cord where VZV is considered to be a possible cause, the appropriate investigations are CSF and peripheral blood PCR for VZV DNA and measurement of anti-VZV IgG in the CSF [27] (Level C).

*Cytomegalovirus*

PCR has been shown to be a rapid and reliable investigative tool for diagnosing CNS infections caused by CMV, especially in AIDS patients [29,30]. Neurological complications caused by CMV are particularly common in immunocompromised individuals, especially those with HIV infection, and include encephalitis and ventriculitis, acute polyradiculopathy, myeloradiculopathy, peripheral neuropathy, and retinitis [29,31]. Numerous studies have been carried out confirming the diagnostic utility of CSF PCR for detecting CMV infection, and the consensus is that this technique has a very high sensitivity and specificity, the levels varying somewhat according to the study and the number of cases tested [8]. (Class II). For example, values of 92% and 94% for sensitivity and specificity, respectively, for CSF PCR were reported in one study [32], and another which compared CSF PCR and brain biopsy results in diagnosing CNS lesions in a smaller number AIDS patients suggested a 100% sensitivity [33]. As CMV PCR is able to quickly diagnose and distinguish a CMV infection from other HIV-associated neurological conditions, it is a valuable technique [8]. Therefore, the use of CMV PCR on the CSF of patients with suspected CMV-associated neurological disease is recommended (Level B). The use of quantitative CMV PCR has also been described to determine viral load that may correlate with disease severity and monitor the efficacy of anti-viral therapy [29,31].

*Epstein–Barr virus*

PCR has been of value in some patients in identifying EBV as the likely causative agent of various neurological diseases, especially in immunocompromised patents such as those with HIV infection who are particularly at risk from developing primary CNS lymphoma [34]. The range of neurological conditions associated with EBV is wide including encephalitis, aseptic neuritis, cerebellar ataxia, myelitis, and several peripheral nerve disorders including various types of acute radicultis, radiculoplexopathy, acute autonomic neuropathy, Guillain–Barre syndrome, and cranial neuropathies [8,35]. Our knowledge is mainly based on a few case reports of inmmunocompetent patients (Class IV), but if a patient presents with any of these disorders in the absence of an obvious cause, it is wise to carry out EBV PCR on the CSF (Level C) because this may indicate or prove the viral cause. In AIDS patients with a suspected CNS lymphoma, the sensitivity of CSF EBV PCR in identifying this is very high, at 97% in one study [36], and almost 100% overall [33,34], and with a 98.5% specificity [34]. Quantitative PCR can also be used in such patients to predict the risk of developing non-Hodgkin CNS lymphoma and for monitoring the effects of chemotherapy [8,9].

*Enteroviruses*

Enteroviruses (EV) are RNA viruses that are major human pathogens and include the subgroups poliovirus, coxsackievirus, and echoviruses [37]. These viruses produce disease in children more often than in adults, and the disease spectrum includes a non-specific febrile illness, aseptic meningitis, and encephalitis. A chronic meningoencephalitis may also occur in

 

immunocompromised patients. The use of EV PCR on the CSF of patients suspected of having an EV infection has been reported as being highly effective both in terms of accurate diagnosis and improved patient management. Reverse transcription (RT)-PCR for EV provides a rapid and very accurate diagnosis of an infection in <24 h and much more quickly than is possible with standard viral culture [38,39] (Class II). Different PCR methodologies have been used for diagnosis, but it had been apparently well established from many studies that the technique is highly sensitive and specific. For example, one report suggested, based on a number of previous reports, an overall sensitivity of EV RT-PCR in the CSF of 86–90% and a specificity of 92–100% [37]. Estimates of about 95% for both of these values were thought to be very likely [40]. However, more recent evidence has called into question the high sensitivity of CSF PCR for diagnosing CNS EV infection. Thus, it was found that of 16 patients with EV71 infection of the CNS during two outbreaks in the US, only five (31%) of them did CSF PCR for EV yield positive results [41]. PCR of specimens from the respiratory and gastro-intestinal tracts yielded higher results than did CSF. While only a small sample size, this study emphasizes that caution now needs to be exercised in interpreting CSF PCR for EV because of the real possibility of false negative results. The results of ongoing more extensive studies in EV infections will be of great interest.

In any patient, child, or adult, who presents with the neurological features of meningoencephalitis prompt analysis of the CSF for EV, using PCR is recommended (Level B). While most CSF samples are likely to be negative, it is a neurological diagnosis that should not be missed especially because patients may be potentially treated with the antiviral agent pleconaril. The use of enteroviral RT-PCR in identifying children with aseptic meningitis, most of which have been hospitalized, has had an important impact on patient management both in terms of shortened patient stays and making significant health care savings [42].

*JC Virus*

JC Virus (JCV) is a polyoma virus that is the causative agent of progressive multifocal leukoencephalopathy (PML), a demyelinating infection of the CNS that occurs mainly in immunocompromised individuals, primarily those with AIDS [43] and recently also associate with natalizumab therapy in multiple sclerosis patients [44]. The use of CSF PCR to detect JCV DNA is now the established and routine method of diagnosis in PML and has superseded brain biopsy [45–47]. The specificity of CSF PCR for this condition is excellent at 98.5–100% [48,49], although the sensitivity of the technique is lower in the region of 50–82% [50,51] (Class II). In a patient suspected of having PML, a CSF specimen should be analyzed for JCV DNA using PCR (Level B). In cases where the PCR is negative in such a patient, a brain biopsy should be seriously considered to obtain a definitive diagnosis. Several studies have also used quantitative PCR for JCV DNA in the CSF to determine how viral load correlates with clinical parameters in patients with PML. For example, higher CSF JC virus loads have been found to be associated with shorter survival times [46,51], 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 [53].

*Human immunodeficiency virus*

Although a diagnosis of HIV will have already been made on patients' peripheral blood, quantitative PCR to measure the CSF viral load has been a valuable tool in assessing neurological involvement in HIV infection such as HIV-associated dementia and encephalitis [54]. High HIV RNA levels in the CSF correlate with the likelihood of developing, presence, and severity of neurological disease during HIV infection [9] and can also be used to monitor therapy as the CSF viral load decreases markedly following highly active antiretroviral therapy (HAART) [55].

*Human T-cell lymphotropic virus-1*

Human T-cell lymphotropic virus-1 (HTLV-1) is strongly associated with tropical spastic paraparesis and HTLV-1-associated myelopathy. PCR has been shown to have a role in the diagnosis of these conditions. For example, 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 [56]. One overview suggested a sensitivity and specificity of CSF PCR for such neurological diagnosis as 75% and 98.5%, respectively [40]. Very recently, two qualitative real-time PCR assays for HTLV-1 and 2 performed on 318 patients' peripheral blood samples had a sensitivity and specificity of 99.4% and 98.5%, respectively, when compared with Western blot analysis [57]. An evidence class of III overall for these studies with a Level C recommendation for PCR for diagnosis seems reasonable given the current number of studies.

Recommendations for use of PCR in CNS viral infections are summarized in Table 1.

**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 Encephalitis | 96% and 99% [15] | Class 1 Level A<br>May be false negatives during first 3 days |
| Varicella-Zoster virus (VZV) | 80% and 98% [25] | Class III Level C<br>CSF anti-VZV IgG more sensitive than PCR in VZV vasculopathy |
| Cytomegalovirus (CMV) | 92% and 94% [32] | Class II Level B<br>Quantitative PCR may also be clinically useful |
| Epstein–Barr Virus (EBV) | 97–100% and 98.5% [33,34,36] | Class IV Level C<br>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<br>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<br>Combination of CSF PCR and anti-HTLV-1 antibody index useful in diagnosis |

## Bacteria

PCR-based assays for diagnosis of bacterial meningitis have been available for the past two decades. There are several advantages of PCR-based diagnosis of bacterial infections based on the analysis of CSF in patients with neurological infections. Conventional diagnostic methods, for example, direct microscopy, culture, and CSF-immunology, are often wanting in terms of timely diagnosis of neurological infections. In many cases, direct microscopy may be negative or non-diagnostic, because patients might have received prior antibiotics or the sample was insufficient or degraded. Results of conventional culture are usually not available for 48 h. Culture may also be rendered negative by prior empirical antibiotic therapy and could take several weeks or months for slow growing or fastidious micro-organisms like Mycobacteria. CSF-immunology may not always distinguish between an active infection and previous exposure or partially treated remote infection. The PCR results are usually available well within 24–36 h for common bacterial infections and utilize low volume of CSF ($\geq$1 ml) for analysis. Indeed, in one study, the time needed for the entire process from DNA extraction to the end of real-time PCR was only 1.5 h [58].

There are several molecular methods that can be employed for diagnosis of bacterial infections by PCR, and a detailed analysis of these techniques is beyond the scope of these guidelines. Very briefly, PCR methods may employ one of the several available techniques, for example, nested or semi-nested PCR with hybridization and sequencing, or use of universal primers and restriction endonuclease enzyme digestion. Probe-based real-time PCR is often preferred when using a multiplex PCR for simultaneous detection of different specific target sequences. Broad-range bacterial PCR is based on use of primers that recognize conserved regions of the genes encoding for eubacterial 16S ribosomal RNA (rRNA). In general, in house nucleic acid amplification methods are considered less reliable and specific than commercially available tests because of its high variability and are not recommended for diagnostic use.

The relative advantages of different PCR methods in rapid diagnosis of bacterial CNS infections have not been evaluated systematically by many prospective and controlled studies. The broad-range bacterial PCR combined with sequencing may be of particular advantage in rapid diagnosis and identification of the etiologic agent in community acquired bacterial meningitis [59–61]. However, real-time quantitative multiplex PCR is considered a highly sensitive technique for fast identification of a causative pathogen of bacterial meningitis [62,63] and can detect as few as two copies of *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Escherichia coli*, 16 copies of *Listeria monocytogenes*, and 28 copies of group B streptococcus [58] whereas the sensitivity for broad-range 16S rRNA-based PCR was about 10–200 organisms per ml of CSF [60,64]. Nested approach is likely to be superior in detecting meningeal infections with Borreila, Listeria, or Mycoplasma because of the low numbers of bacterial DNA and relatively few copies of 16S rRNA gene in the CSF [59].

 

### Acute meningitis

Currently available PCR methods detect *Hemophilus influenzae*, *N. meningitidis*, *S. pneumoniae*, *L. monocytogenes* in CSF and have a sensitivity of 87–100% and specificity of 98–100% [65]. Presently, quantitative multiplex RT-PCR appears to be the preferred technology for the detection of common pathogens of acute bacterial meningitis. Several quantitative PCR assays for the detection of *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* were developed over the years, and recent publications confirm the validity of multiplex detection of specific target DNAs of these common pathogens in the CSF [58,62,63]. The positive yield of detection rate with the PCR is significantly better than that with the conventional cultures in patients with prior antibiotic therapy. PCR-based detection of bacterial pathogens is also considered more sensitive than culture in patients with ventricular catheters and suspicion of nosocomial meningitis. In one study of ventricular-catheter-associated bacterial meningitis, nearly 50% (42 of 86) specimens were negative as assessed by culture but positive by PCR; there was no positive culture in patients with negative PCR results suggesting that negative PCR result is predictive of the absence of infection [66]. The positive predictive value of broad-range PCR is 98%, and the negative predictive value is 100%; in other words, negative bacterial PCR assay virtually excludes the diagnosis of acute bacterial meningitis [67].

*Recommendation*

For reasons of high inter-assay variability and low specificity, in house nucleic acid amplification methods for diagnosis of bacterial infections in CSF are deemed unreliable and should not be used in clinical practice (Class IV Grade C). The robustness of various commercial PCR tools that are currently available and the choice of uniplex or multiplex quantitative RT-PCR for appropriate levels of diagnostic specificity and sensitivity are presently unclear and remain to be defined by field tests and comparative studies (Class IV Grade C).

### Chronic meningitis

Securing an early and accurate microbiological diagnosis of tuberculous meningitis (TBM) remains a major challenge, both in adults and in children, in view of the low sensitivity of smear positivity (<10%) and the prolonged period of culture time required. Quantitative RT-PCR has been shown to substantially increase diagnostic yield in TBM. Overall, the reported sensitivities of PCR-based tests for *Mycobacterium tuberculosis* in CSF samples range from 46% to 66% and specificities from 97% to 99% [68], with ranges as wide as 2–100% for sensitivity and 75–100% for specificity, have been reported in different studies. Higher yield of positive result in quantitative RT-PCR test has been observed after using CSF filtrate as compared with CSF sediment [69], and a novel RT-PCR-based rapid detection method (Xpert MTB/RIF assay), that is relatively easy to use, has shown great promise in identifying drug-resistant Mycobacteria in pulmonary tuberculosis [70]. However, its value in the detection of drug-resistant mycobacterial strains in CSF samples is yet to be tested.

PCR-based methods are as prone as conventional cultures for cross-contamination, and the diagnostic specificity of PCR-based diagnosis of TBM may be compromised in endemic areas [71]. Consequently, caution must be exercised in the interpretation of a PCR-based result in TBM that may be falsely positive while a negative CSF PCR result does not exclude the diagnosis of neurotuberculosis in an appropriate clinical setting when supported by the CSF and imaging data. In contrast to acute bacterial meningitis where CSF rapidly becomes sterile after antibiotic therapy and bacterial DNA may not be detectable beyond 8 h of treatment [72], there is some evidence that Mycobacterial DNA may persist for up to a month in CSF after starting therapy and repeating quantitative RT-PCR test in successive CSF samples for *M. tuberculosis* may aid to diagnosis even if initial PCR result is negative [73].

CSF PCR for Borrelia is probably useful as a diagnostic test only in very early stages of Lyme neuroborreliosis and is not recommended as a diagnostic test for chronic Lyme disease or measure treatment response as a follow-up [74]. PCR on skin biopsies may become useful in the diagnosis of early Lyme borreliosis in patients with atypical forms of erythema migrans.

*Recommendation*

The diagnostic yield of PCR in CSF is 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 summary, PCR-based techniques offer a rapid and sensitive diagnosis of several common bacterial CNS infections. This may be important in patients with suspected acute bacterial meningitis to confirm diagnosis and tailor the antibiotic therapy. In cases of suspected meningococcal meningitis, an early microbiological diagnosis is necessary for epidemiological reasons to prevent secondary cases. However, PCR-based diagnostic tools should be used as an adjunct

rather than a substitute for current methods of bacteriological diagnosis by conventional staining and culture. In the future, Gram stain-specific probe-based real-time PCR using 16S rRNA may allow simultaneous detection and discrimination of clinically relevant Gram-positive and Gram-negative bacteria directly from blood samples that might provide a rapid diagnosis of bacterial infections [75]. Loop-mediated isothermal amplification is a novel nucleic acid amplification technique distinct from PCR, which amplifies DNA under isothermal conditions (63◦ C) and the result can be interpreted visually [76], but its role in the rapid diagnosis of bacterial infection in CSF for neurological infections awaits further research.

*Recommendations*

Commercially available and standardized quantitative RT-PCR is a valuable adjunct for diagnosis of bacterial meningitis and is recommended for routine use in CSF samples (Class II, Grade A) of patients with suspected bacterial meningitis. However, direct microscopy and culture remain the gold standard of microbiological diagnosis of bacterial infections of central nervous system where feasible and current range of diagnostic bacterial PCR tests do not replace them (Class II Grade A).

## Parasites

Molecular diagnostic testing for parasitic infections includes conventional, real-time, consensus and degenerate PCR and, even, micro-array analyses. PCR-based techniques are capable to detect and characterize genetically different species and population variants of protozoan and, at least a few helminthic parasites. However, PCR, being capable to directly detect parasite DNA/RNA is highly sensitive and specific, is still too expensive to be used routinely in geographic areas where most of the parasites occur endemically.

In immuno-compromised patients, indirect diagnostic methods (serology for antibody detection, etc.) have a very low sensitivity. Thus, in this group of patients, optimization of diagnostic sensitivity by direct detection of the pathogen's DNA or RNA is essential for earliest possible diagnosis and optimization of therapeutic management.

The diagnosis of a parasitic infection of the CNS rests upon clinical signs and symptoms, clinical history, travel history including geographic exposure [77,78] and, finally, on laboratory techniques [79]. The primary tests, which are currently used to diagnose a majority of parasitic diseases, in particular, in tropical, resource-poor areas, have virtually not changed throughout the past decades, light microscopy still being the diagnostic mainstay. Indirect methods, that is, serology, can frequently not distinguish between past, latent, reactivated, or acute infection and are of little use in ascertaining therapy response or for prognosis. Beside highly specific tests to detect antigen, for example, rapid antigen detection system (RDTS) [80], luciferase immune precipitation system (LIPS) [81], molecular-based approaches, in particular PCR [77], loop-mediated isothermal amplification (LAMP) [82], real-time (RT) PCR [83], and luminex technology [84] have shown a high potential for use in diagnosing parasitic infestations with increased sensitivity and specificity [77].

The molecular-based approaches in diagnosing CNS infections and infestations by parasites (protozoa and helminths) having the potential to cause CNS disease, RT-PCR, loop-mediated isothermal amplification (LAMP), and luminex xMAP technology are discussed.

### Molecular-based diagnostic tests in protozoal infections of the CNS

Table 2 [80,84–122] lists the protozoa that have the capacity to invade the CNS, causing neurological disease and details the molecular-based techniques which have, at least in theory, the potential to support the diagnostic sensitivity and specificity. It must be mentioned that only in cerebral toxoplasmosis, PCR and RT-PCR have been introduced into the routine diagnosis in immuno-compromised patients. The other molecular-based assays are still experimental diagnostic techniques and have not yet replaced serology or direct proof by light microscopy.

Bearing in mind all these newly developed diagnostic techniques based on molecular approach, it must be stressed—again—that so far both in the field work in resource-poor countries, and, similarly, also in European University hospitals, these techniques are not yet ready to be used on a routine basis, they cannot yet replace fully (with the exception of toxoplasmosis) the light microscopy or, in part, serology.

### Molecular-based diagnostic tests in helminthic infestations of the central nervous system

Table 3 [84,123–144] lists the CNS helminthoses, their neurological manifestation, and the modern molecular-based diagnostic techniques. It needs to be stressed even more that in helminthic diseases, the direct visualization or detection of the helminths, either the adult worm, the larval stage, or the eggs, be it in body fluids or biopsied material, still represents the golden standard of diagnosis.

 

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

*Recommendations*

Microscopy and serology show many limitations in the diagnosis of protozoal infections or helminthic infestations of the CNS. Molecular techniques have enabled parasitologists and neuroinfectiologists to use the gene amplification methods to establish the diagnosis from any kind of body fluids, that is, also the CSF, or biopsy material. Conventional PCR has been supplemented by nested and multiplex PCR as well as real-time PCR for the detection of several parasitic infestations and infections, respectively. Recently, even more modern techniques as loop-mediated isothermal amplification (LAMP) and luminex-based assays have been proposed as possible diagnostic techniques in parasitic diseases of the nervous system. As these techniques allow the detection of infestations or

**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]<br>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]<br>Loop-mediated isothermal amplification [128] | III | |
| Schistosoma spp. | Space-occupying granuloma intracerebral and spinal space- occupying granuloma | PCR [129,130]<br>Real-time PCR [131,132]<br>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]<br>Real-time PCR [135]<br>Pentaplex-real-time PCR [136]<br>High throughput multiplex PCR and probe-based detection with luminex beads [87]<br>Duplex-real-time PCR [137] | II | B |
| *Taenia solium* – larval stage: *Cysticercus cellulosae* | Neurocysti-cercosis (Space-occupying, cystic intracranial lesions, epilepsy) | PCR [138,139]<br>Nested PCR [140]<br>Semi-nested PCR [141]<br>PCR amplified DNA sequences targeting *T. solium* mitochondrial cox1 gene and cob gene [142]<br>Loop-mediated isothermal amplification [143] | II | B |
| *Toxocara canis* (cati) | Larva migrans visceralis (cerebralis, intracranial granuloma, vasculitis) | PCR [144] | IV | |

infections from samples with very low burden of parasites, these molecular-based approaches offer higher sensitivity and enhanced specificity compared with existing diagnostic tests. These techniques have been established, at least in part, as the reference diagnostic tool in European laboratories, and they are used for research purposes in tropical areas. However, they are far from having become daily routine in the diagnosis of parasitic infections and infestations of the CNS in resource-poor countries where history, clinical signs and symptoms, and direct light microscopy still remain the mainstay of diagnosing CNS parasitoses.

## Fungal infections

CNS infection can be the consequence of disseminated infection or CNS confined. The incidence of invasive fungal infections is rising with the increasing number of immunocompromized individuals. Positive cultures together with microscopy, antigen/antibody testing in serum, and CSF are the diagnostic 'gold standard'. Slow growth of fungi in culture, cross-reactivity in case of antigen detection, and dependence on the demonstration of an antibody response or even by the failure to mount an adequate immune response are major drawbacks [145].

### Histoplasmosis (*Histoplasma capsulatum*)

The clinical spectrum of histoplasmosis, the most common endemic mycosis in Europe [146], ranges from asymptomatic infection to life-threatening illness. Chronic meningitis is the most frequent CNS manifestation, whereas cerebral or spinal masses and encephalitis are less common.

Fungal culture is the gold standard diagnostic test in non-CNS manifestations, but it may take up to

 

6 weeks. CSF cultures usually do not yield growth [147]. Histoplasma capsulatum antigen and antibodies can be determined by different methods in CSF and are used to establish diagnosis. However, cross-reactivity with other dimorphic fungi and Cryptococcus spp in up to 50% needs to be taken into account [148]. In a study of 14 episodes of Histoplasma meningitis, all samples were positive for Histoplasma antigen, whereas antibodies were only present in 9 of 13 samples tested [149]. A limited number of studies evaluated the value of PCR in CNS histoplasmosis (class IV) [147,150,151], but there is no commercial kit available for routine use.

#### Coccidioidomycosis (*Coccidioides immitis*)

Chronic basal meningitis is the most common coccidioidal CNS manifestation. Occasionally, CNS coccidioidomycosis presents as meningoencephalitis or intracranial mass lesion.

Coccidioides sp. grows in culture within 2–5 days [152] but is isolated from CSF only in a third of patients with CNS manifestations [153], making diagnosis of isolated coccidioidal CNS infection challenging and may rely on antibody testing in serum and CSF. CSF antibodies can be detected in up to 70% of patients with meningitis during the initial analysis, and in the majority of cases later [145]. Currently, there are no standardized CSF antigen detection methods [154], and no PCR is available for commercial use [151].

There are 2 class IV anecdotal studies using PCR for the diagnosis of coccidioidal CNS disease with conflicting results [155,156].

#### Blastomycosis (*Blastomyces dermatitidis*)

CNS manifestations of blastomycosis are rare, mostly cranial/spinal epidural abscess, meningitis, and brain abscess in AIDS patients. Definitive diagnosis of blastomycosis requires growth of the organisms in culture. CSF culture is rarely positive, and only stereotactic brain biopsy may be diagnostic [145,154]. While CNS blastomycosis may be diagnosed by presence of antibodies in serum and CSF, cross-reactivity with other fungi needs to be considered. Chemiluminescent DNA probes have been developed but are neither standardized nor available for commercial use [152]. Yeast forms may be seen on cytological examination of CSF [154].

#### Cryptococcosis (*Cryptococcus neoformans/gattii*)

Chronic basal meningitis is the most frequent CNS manifestation of cryptococcal disease causing subacute dementia or visual symptoms. Culture alone is generally not the method of choice. The diagnostic mainstay is antigen detection with >90% sensitivity and specificity [145,154]. This test in CSF can be positive early in infection. Microscopic examination of CSF using India ink stain is diagnostic in up to 80% of AIDS patients and about 50% in non-immunocompromized [145].

There are five studies using PCR for the diagnosis of CNS cryptococcosis, three with evidence class III and two evidence class IV. The first evaluated different diagnostic tests (culture, microscopy with India ink and Gram staining, latex agglutination test, EIA, and PCR) in 46 CSF samples with evidence for cryptococcal CNS infection by one of the methods [156] and 40 control CSF samples from patients with other CNS infections. PCR and EIA had a sensitivity and specificity of 100%. A 100% sensitivity was detected for culture, India ink, and Gram stain, whereas specificities were lower (84.8%, 93.5%, and 95.7%, respectively). Latex agglutination test had 100% sensitivity but lower specificity (86.7%). Another study reported similar findings [157]. The third class III study compared culture, India ink test, and PCR in CSF of 56 patients with cryptococcal meningitis and 16 patients with meningitis of other origin [158]. While all three tests had specificities of 100%, the sensitivity rate was highest for PCR (92.9%), followed by India ink test (85.7%) and culture (76.8%). A false negative rate was found for PCR (20%) and India ink test (33.3%). The two class IV evidence studies are listed in Table 4 [159,160].

#### Candidiasis (*Candida albicans* and other *C. species*)

Candida species are the fourth leading cause of fungal bloodstream infections and associated with a mortality rate of up to 50%. CNS Candidiasis can develop in the setting of disseminated candidiasis with microabscesses of the brain parenchyma or as candida meningitis in association with a foreign body (e.g. catheter, ventricular shunt) or other CNS invasive procedures (e.g. surgery).

Blood cultures are considered the gold standard for routine diagnosis of invasive candidiasis but are time-consuming. In CSF studies, only 17% of stains and 44% of routine cultures identified the pathogen in chronic Candida meningitis [161]. Serological diagnosis of CNS candida infection, either by antigen detection or by antibody determination, has not been validated. Detection of the Candida antigen mannan in CSF has been tested in a few cases [162]. PCR protocols for detection of candidal infection have been described and require probes for

**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[a] | 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 (2002) | 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[a] | 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)

**Table 4** *(Continued)*

| | 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mucormycosis | – | 179 | Germany (2006) | IV | 1 | Sinusoidal tissue | | | | | 1 |
| | | 180 | Germany (2005) | IV | 23 | Paraffin-embedded tissue | | 23 | | | 14 |

+ PCR, positive PCR; –, no recommendation.
[a]See conclusions of chapter fungal CNS infections.

different subspecies [163]. PCR may also assess mutations associated with resistance to antifungal medication.

There are only three class IV evidence studies for PCR diagnosis of CNS candidiasis. [164–166].

### Aspergillosis (*Aspergillus fumigates* and other species)

There are numerous species of aspergillosis recognized, but most cases of CNS infection are attributed to *A. fumigates*, *A. flavus*, *A. terreus*, and *A. versicolor*. Major CNS manifestations include hemorrhagic infarction, abscess, and meningitis; less frequent are mycotic cerebral aneurysm and granuloma.

Aspergillus species are ubiquitous pathogens, and hence, culture is insensitive and diagnosis requires non-culture-based methods. Two antigen assays, the [1,3]-beta-D-Glucan assay (sensitivity 87%), a relatively non-specific assay and the galactomannan assay (>95% sensitivity and specificity), an Aspergillus-specific antigen, are commercially available and used in clinical routine for blood specimen [152]. In a few cases, the latter assay has been also used for CSF samples [167–169]. PCR was compared with the two antigen assays in 33 patients with invasive pulmonary aspergillosis [170]. Sensitivities of 79% (PCR), 58% (galactomannan), and 67% ([1,3]-beta-D-Glucan), and specificities of 92%, 97%, and 84%, respectively, were reported in peripheral blood. Positive PCR findings preceded those of galactomannan (2.8 ± 4.1 days) and [1,3]-beta-D-Glucan (6.5 ± 4.9 days). Other studies indicate that a combination of the galactomannan assay and PCR may improve diagnosis. Two studies report that the sensitivity of PCR testing for Aspergillus is limited during antifungal treatment [171,172].

There are two class III evidence studies related to PCR diagnosis of CNS aspergillosis. The first compared EIA, latex agglutination (LA) test and PCR in CSF of five patients with pulmonary aspergillosis and focal neurological signs but negative CSF cultures, and in 11 controls [173]. PCR was positive in all five patients butEIA or LA only in 4 (80%). CSF PCR was negative in all controls. In the second study, PCR for diagnosis of *Aspergillus meningitis* was investigated with 26 CSF samples obtained from a single patient with a proven Aspergillus fumigates infection [167]. Galactomannan was detected in the CSF 45 days before a culture became positive, and Aspergillus DNA was detected 4 days prior to culture.

There are five smaller studies with class IV evidence [171,174–177], which are listed in Table 4.

#### Mucormycosis/zygomycosis (Mucor, Rhizpous, Rhizomucor, Absidia, Cunninghamella, Apophysomyces, Saksenaea)

Mucormycosis is an acute and aggressive fungal infection, which can develop as isolated cerebral mucormycosis (16%), extension to the brain from rhinocerebral mucormycosis (69%) or via the hematogenous route (15%) [178].

There are different methods for making a diagnosis of zygomycosis including histology, culture, and PCR. The diagnosis is mostly made by a combination of histology and culture [179].

There are only two reports on the diagnosis of rhinocerebral zygomycosis by PCR (class IV evidence): first, a case of rhinocerebral zygomycosis that was diagnosed by PCR of a sinusoidal biopsy specimen but no culture was performed [180]. The second is a study of PCR in paraffin wax embedded tissue and histological confirmation [181]. Amongst were paranasal sinusoidal and brain tissue and PCR was positive in 14 out if 23 samples.

#### Conclusion

The diagnostic advancement by PCR seen in other CNS infections has not moved into the foreground for fungal CNS infections (Table 4). This is in part because of the relative infrequency of fungal CNS infections and to the lack of a gold standard against which studies evaluating sensitivity and specificity of advanced methods can be verified. The usage of 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 studies reporting the feasibility of CSF PCR for evaluating CNS manifestations by Histoplasma, Coccidioides, and Candida, and of tissue for CNS mucormycosis. However, we do not identify enough evidence to recommend the use of PCR as a routine diagnostic tool in these cases.

## Recommendations

The use and ability to provide diagnosis of neurological infection by PCR varies according to the group of pathogens. No doubt: the main contribution of this technology is to the diagnosis of infections caused by viruses followed by bacterial infections of the CNS with the notable exception of tuberculous meningitis.

The efficacy of this tool for the diagnosis of both protozoal infections and helminthic infestations has also been established in many instances. Unfortunately, the molecular technology at large, including PCR, is far from becoming routine in resource-poor countries where such infections are prevalent.

As for fungal infections, despite their importance in the context of the immune-compromised host, there is not enough data to recommend the routine use of PCR. More clinical research is required to test and eventually confirm its role in this group of infections.

 

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