folia medica

Folia Medica 64(5):709-715
DOI: 10.3897/folmed.64.e72257

Review

Current Methods for Microbiological Diagnosis of Acute Central Nervous System Infections

Yordan Kalchev[1,2,3], Marianna Murdjeva[1,2,3]

[1] *Department of Medical Microbiology and Immunology "Prof. Dr. Elissay Yanev", Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*

[2] *Laboratory of Microbiology, St George University Hospital, Plovdiv, Bulgaria*

[3] *Research Institute at the Medical University of Plovdiv, Plovdiv, Bulgaria*

Corresponding author: Yordan I. Kalchev, Department of Medical Microbiology and Immunology "Prof. Dr. Elissay Yanev", Faculty of Pharmacy, Medical University of Plovdiv, 15A Vassil Aprilov, 4002 Plovdiv, Bulgaria; Email: yordan.kalchev@mu-plovdiv.bg; Tel.: +359 898 431919

Received: 27 July 2021 ♦ **Accepted:** 4 October 2021 ♦ **Published:** 31 October 2022

Citation: Kalchev Y, Murdjeva M. Current methods for microbiological diagnosis of acute central nervous system infections. Folia Med (Plovdiv) 2022;64(5):709-715. doi: 10.3897/folmed.64.e72257.

Abstract

The incidence of infections affecting the central nervous system has increased in recent years, making neuroinfections a current global health problem. The central nervous system is quite well protected from the external and internal environments, although it is susceptible to infection by a wide variety of pathogens. The etiological diversity further complicates the management of such infections because it is important to identify correctly the specific cause in order to choose the most appropriate antimicrobial therapy. Diagnosis is made not only based on clinical and epidemiological data but also on the results of clinical laboratory and microbiological examination of cerebrospinal fluid. This article aims to review current microbiological methods in the diagnosis of acute central nervous system infections and help healthcare providers to recognize their advantages and limitations in order to manage their patients appropriately.

Keywords

CSF culture, CNS infections, direct microscopy, latex-agglutination test, multiplex PCR, neuroinfection

INTRODUCTION

The incidence of infections affecting the central nervous system (CNS) has increased in recent years despite the remarkable advances in infection control and public health, such as the introduction of vaccine prophylaxis and the development of new antibiotics.[1] This is largely dependent on the growing number of immunocompromised individuals owing to the rise in oncological and autoimmune diseases, the widespread use of a variety of immunosuppressive drugs, as well as the expansion of HIV infection. According to data from the National Center for Infectious and Parasitic Diseases (NCIPD) in Bulgaria, the main cause of mortality among acute infectious diseases in the country for 2018 was neuroinfections, accounting for 40% of all deaths. The continuous development of antimicrobial resistance is another factor that adversely affects the control of patients with neuroinfections, which severely limits the treatment options and the choice of empirical antimicrobial therapy.

Pathogens associated with CNS infections are diverse including viruses, bacteria, fungi, and parasites. These microorganisms differ greatly by geographical region, country, age, immunological reactivity of the macroorganism, and levels of the vaccine prophylaxis. This etiological diversity is a real challenge and makes it very difficult both to identify the specific cause and to guide the most appropriate therapy. Therefore, it is recommended to start empirical antibiotic therapy before obtaining results from the microbiological analysis.

Microbiological evaluation of acute CNS infections

For optimal results, it is of great importance to collect cerebrospinal fluid (CSF) specimens and transport them properly to the laboratory of microbiology.[2,3] It is advised that clinical samples should be taken before the initiation of antimicrobial therapy, but on no occasion should treatment be delayed to obtain the CSF. WHO recommends processing CSF samples within 1 hour of collection.[3]

The most commonly used methods for microbiological diagnosis include direct microscopic examination and CSF culture with subsequent isolate identification.[3,4] Although these approaches are widely used in diagnosing patients with neuroinfections, they have significant limitations and may not always provide a rapid and accurate etiological diagnosis. In order to overcome the limiting factors of the conventional methods for microbiological diagnosis, other tests such as latex-agglutination test (LAT) and nucleic acid amplification techniques were introduced.

Direct microscopy and staining

In 1884, while working in the Berlin morgue under the direction of Dr. Friedlander, the Danish bacteriologist Hans Christian Joachim Gram created a new method of staining. In an attempt to find the cause of bacterial pneumonia, Gram noticed that some bacteria, once stained blue with aniline-gentian violet, did not discolour after the subsequent application of ethanol (Gram +), while others lost their colour (Gram −). This is due to structural differences in the bacterial cell wall. A few years later, the German pathologist Carl Weigert modified the procedure by proposing the addition of a second dye (safranine), subsequently staining the already discoloured Gram (−) bacterial cells in red.[5]

Although Gram was modest about his discovery, for more than 130 years, Gram staining, along with CSF culturing, has been the most widely used method for microbiological diagnosis in patients with acute neuroinfection.[5,6] It is not only a fast but also cheap and easy-to-perform technique. However, it is not applicable in patients with viral infections of the CNS. Furthermore, in bacterial neuroinfections, the positive rate varies greatly. According to some authors, it ranges between 60 and 90%, while others report success in Gram stain examination in 24-97%.[4,7]

Given the most common bacterial pathogens associated with neuroinfections and their specific morphological characteristics, visualization of Gram (+) cocci in pairs points to *S. pneumoniae,* Gram (−) diplococci to *N. meningitidis,* Gram (+) rods to *L. monocytogenes,* and Gram (−) polymorphic rods to *H. influenzae.*[1] The presence of Gram (−) rods has been rarely observed in immunocompetent individuals, except in the presence of predisposing factors, and it is usually attributed to enteric bacteria.[8] Besides all this, an experienced microbiologist is needed to correctly interpret these findings. False-positive results of direct microscopic examination may be due to misinterpretation by the observer, as well as contamination of the clinical samples or reagents.[4]

It has been found that the sensitivity of direct microscopy varies significantly with the exact bacterium present. In the highest proportion, Gram staining has been able to establish the aetiology of patients with pneumococcal meningitis (69-93%). The direct microscopic examination could reveal *H. influenzae* in 25-65%, and meningococci in 30-89%. According to some other authors, *H. influenzae* can be found in higher percentage – 86%.[4] If *L. monocytogenes* is present both in adults and children, the sensitivity of the Gram staining is even lower (10%-35%). Only half of the Gram (−) microorganisms could be observed under a microscope.[10]

In a currently not published study on the application of Gram staining in bacterial meningitis, we were able to confirm some of these observations. The overall positivity rate of Gram stain examination we determined was 52.2%. The majority of cases were due to *S. pneumoniae*, which we observed on Gram stain in 90% of all pneumococcal neuroinfections. The method failed to detect culture-positive listerial meningoencephalitis, as well as *H. influenzae* and the majority of the Gram (−) enteric pathogens of CNS infections. We calculated a sensitivity of the direct microscopy with Gram stain of 48% (95% CI 21.8–68.1%) and specificity of 100% (95% CI 95.8–100%).[51]

In addition, the method is highly sensitive to an initiated antibiotic treatment prior to the CSF collection because these drugs can rapidly decrease the number of pathogens presented.[7,9] The sensitivity can be reduced by nearly 20% in CSF samples collected from patients pretreated with antimicrobials when compared to individuals who did not receive any antibiotic therapy prior to the specimen collection.[4,10] This is due to the fact that the likelihood of detecting an etiological agent by Gram stain depends on the concentration of the pathogen in the clinical sample. At concentrations of 10^3 colony-forming units (CFU)/ml and below, the staining is positive only in 25%, at a concentration of 10^3 – 10^5 CFU/ml in 60%, while at a concentration of 10^5 CFU/ml and more, a positive result is observed in nearly 97%.[10] Studies have demonstrated that the CSF can be rapidly cleared of bacteria after parenteral use of antibiotics sterilizing the sample after 4 hours of application in pneumococcal and 2 to 6 hours in meningococcal meningitis.[11,12] The reduced number of pathogens may not only be due to the prior use of antimicrobials but also improper storage and transport of the clinical samples to the laboratory of microbiology.

Other staining methods show an even lower sensitivity when compared to Gram stain. Among patients with tuberculous meningitis, where Gram staining is not a choice, even if specific staining methods for acid-fast bacteria are used (such as Ziehl-Neelsen stain or Kinyoun stain), success can be achieved only in 10% – 50%.[13,14]

Notwithstanding, it is reasonable to point out that the method allows evaluating the cellular reaction by observing inflammatory cells and determining their type, if pres-

ent, which sometimes can help differentiate between contamination and true infection.

CSF culture

CSF culture is considered the 'gold standard' in the diagnosis of neuroinfectious diseases, especially in the case of bacterial meningitis. The method is based on inoculation of CSF samples in specific growth media, incubation of the plates at appropriate conditions, and subsequent identification of the colonies, if present. The results are usually available in 24 to 72 hours, depending on the type of microorganism and the identification method available at the laboratory, which is a significant disadvantage in emergency conditions like an acute CNS infection.[2,7,15] The utilization of modern systems like Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) can significantly increase the time to the final identification of the isolate compared to the traditional biochemical method for identification. Even more, CSF culture result is greatly influenced by the transportation of the clinical material to the laboratory and the time to the initiation of empiric therapy. As already mentioned, WHO recommends that cerebrospinal fluid should be delivered to the laboratory within 1 hour of specimen collection, which in real practice cannot always be achieved.[3] Similar to Gram staining, the overall positivity rate of CSF cultures also varies, but in a narrower range (70%-85%), even if it is collected and transported correctly, or collected before the initiation of antibiotic therapy.[3,16] Other authors report a 60-90% positive rate under the same conditions.[7] It has been found that a positive result of culturing is possible only when collecting clinical samples up to 4 hours after the application of antibiotic treatment.[2,17] Other researchers have reported a wider window period with a culture-negative result one day after the initiation of antimicrobial therapy.[18] A study by Etyang et al. compared the recovery rate by direct bedside inoculation of cerebrospinal fluid and the traditional laboratory culture method. The obtained results showed the absence of statistically significant differences when comparing the two approaches. However, significant differences in time were found, with bedside cultures showing growth 5 hours earlier than the conventional laboratory cultures.[19]

Another factor worth mentioning is the use of liquid growth media for enrichment. These are found to slightly increase the sensitivity of the CSF culture but are associated with multiplying contaminants and thus reporting false-positive results without contributing much to the diagnosis.[20-22] It has been reported that when inoculating CSF samples into BACTEC blood culture bottles, a higher detection rate is observed comparing culturing on solid growth media (agars).[23]

A study in the UK of 103 patients with meningococcal meningitis found that only 13% of them had a positive CSF culture.[24] Another research from Nepal of 296 patients under the age of 18 found that only 4.4% of them had a positive culture result.[25] Mycobacterial culture in patients with tuberculous meningitis has a sensitivity of about 40%-50%. The results can be interpreted after 8–10 days only when inoculated in liquid media.[26,27] In another study among patients with the same disease, *M. tuberculosis* was cultured only in 31.2%.[28] Larger amounts of cerebrospinal fluid in the range of 5-10 ml are needed for the cultivation of mycobacteria as well as for fungi.[29]

Cryptococcus neoformans culture is also considered the gold standard, but higher volumes are needed to increase the sensitivity of the method otherwise false-negative results can occur. Furthermore, the fungus growth may require up to 10 days.[30]

Due to the long time required for viral cultures and the low sensitivity, the culture method cannot provide a timely etiological diagnosis of viral pathogens.[15] Therefore, it is not applicable in the emergency of acute CNS infections and it is only of retrospective consideration. The sensitivity of the enteroviral culturing, the most common cause of viral meningitis, ranges between 65 and 75%, and some of them, such as *Coxsackievirus A,* are extremely difficult to be grown *in vitro.*[15,31]

Blood for blood cultures

Given the fact that the haematogenous spread of microorganisms is the most commonly encountered mechanism for CNS invasion and infection, blood samples for blood cultures can help determine the aetiology in neuroinfections. The higher positivity rates are associated with the implementation of automated blood culture systems. However, similar to CSF culturing, blood culture results are also dependent on the exact bacterial pathogen and the use of antibiotics before blood collection can decrease the yield by 20%.[2,50] The reported recovery rate for *S. pneumoniae* from blood samples is 75%, followed by *H. influenzae* (50-90%), and meningococci (40-60%).[2]

Latex-agglutination test (LAT)

LAT is based on the detection of bacterial or fungal antigens directly in the cerebrospinal fluid samples. The test is easy to perform and allows rapid diagnosis within 15 minutes.[7] Despite these advantages, the reliability of this test in identifying the etiological agent of bacterial meningitis has been questioned in recent years due to numerous scientific reports of the low sensitivity of the method, especially in patients pretreated with antibiotics.[32,33] The overall sensitivity of the test varies between 67 and 100%. [7] Similar to Gram stain and CSF culture, the method has pathogen-specific sensitivity. The reported sensitivity in *H. influenzae* varies between 78% and 100%, in *S. pneumoniae* – 59–100%, and *N. meningitidis* – 22–93%.[16] A retrospective study of 176 children pretreated with antibiotics showed that LAT did not identify any causative agent.[32] In patients with a culture-negative result, LAT has a sensitivity of only 7%.[33] In another study of 344 cerebrospinal fluid

samples, the test did not affect either therapy or the course of the disease, and even false-positive and false-negative results were recorded.[34] The method is only applicable to a limited number of pathogens, namely capsule-forming microorganisms associated with neuroinfections, which is another prerequisite limiting its use. Combined tests are available to detect the antigens of these bacteria, as well as for the most common isolate – *S. pneumoniae*. In Australia, a positive LAT for *S. pneumoniae* is an indication for inclusion of vancomycin in the empirical antibiotic therapy.[35,36] We calculated a sensitivity of LAT of 47.8% (95% CI 26.8–69.4%) and specificity of 100% (95% CI 95.9–100%), the same parameters as the direct microscopy.[57]

The detection of CSF cryptococcal antigen has replaced staining with India ink for *C. neoformans* and *C. gattii*, with the test showing sensitivity and specificity over 90%. However, false-positive and false-negative results have been reported, especially in people with HIV-positive status, where the disease is most prevalent.[29]

Enteroviruses do not have a common antigen, which makes it impossible to create an antigen-antibody-based test for their detection. Specific IgG antibodies can be sought for HSV, but they can be detected after 10-12 days, making this approach inapplicable given the need for a rapid etiologic diagnosis.[15,37]

A negative LAT test does not rule out the presence of the pathogen in the clinical material, and possible false-positive results may become the basis for inadequate therapy.

Polymerase chain reaction (PCR)

In recent years, there has been a revolution in the microbiological diagnosis of neuroinfections with the introduction of the molecular genetic techniques of monoplex or multiplex PCR assays.[38] These methods are used to detect nucleic acids (DNA or RNA) of viruses, bacteria, fungi, and parasites in CSF specimens. They have been developed in order to overcome many disadvantages of the conventional diagnostic methods in patients with acute CNS infections, and now PCR-based techniques are a popular tool for microbiological diagnosis.[39] The use of multiplex PCR showed a sensitivity of 100% and a specificity of 98.2%, a positive predictive value of 98.2%, and a negative predictive value of 100%.[7,41] Corless et al. found that susceptibility again depends on the etiological agent, with 92% for *H. influenzae,* 100% for *S. pneumoniae,* and 88% for *N. meningitidis,* with the specificity for these three pathogens being 100%.[42] Another study reported different results – a sensitivity to *H. influenzae* of 88%, to *S. pneumoniae* of 92%, and *N. meningitidis* – 94%. The determined specificity for these pathogens was once again 100%.[43] Ni H et al. also report that the sensitivity and specificity of the method is 91% among patients with meningococcal meningitis.[40]

The approach remains less affected by prior use of antimicrobial drugs compared to the direct microscopy and culturing of CSF specimens.[36] A study showed a sensitivity of the method of 89% on days 1-3 of the start of antibiotic therapy, 70% on days 4-6, and 33% on days 7-10.[18]

In Bulgaria, there are no systematic studies on the role of mPCR for the rapid diagnosis of acute meningitis/meningoencephalitis. However, there is a significant experience in the PCR diagnosis of meningitis caused by *N. meningitidis, H. influenzae,* and *S. pneumoniae* by a study group of scientists from NCIPD, Sofia and Stara Zagora.[44,45] Although serotypes of some microorganisms and resistance genes can be detected by PCR, CSF culture still remains the main method for in vitro testing of the antimicrobial susceptibility as well as for subsequent serogrouping and serotyping. In this country, Levterova V. and Simeonovski I. have the expertise to use PCR assay in serotyping of pneumococci and meningococci.[44] In addition, our experience with multiplex PCR showed a sensitivity of 100% (95% CI 81.5–100%) and specificity of 100% (95% CI 96.1–100%) for the bacteria and fungi, included in the panel, and a sensitivity of 88.2% (95% CI 63.6–98.5%) and specificity of 100% (95% CI 96.2–100%) for the viruses included in the spectrum of the test.[57] However, it is worth mentioning that despite the high diagnostic value of mPCR, the circulation of pathogens not included in the test panel in the Plovdiv region was significant (30.4%). It can be a reason for false-negative results and mPCR negative results should be carefully evaluated.[57] This in turn cannot restrain the need for CSF culturing in PCR-negative patients.

For the detection of enteroviruses, genetic methods have also shown significantly higher specificity and sensitivity when compares to cell cultures.[46,47] Therefore, RT-PCR searching for the 5' non-coding regions of viral RNA is recommended, and serological methods and viral in vitro cultures should be avoided for routine diagnosis. In most cases, viral RNA is detected in cerebrospinal fluid in patients with meningitis, but this finding is erratic in individuals with rarer enteroviral encephalitis. The virus is excreted longer in stool samples and can be found there, but this does not always contribute to proving the etiological link to CNS involvement.[48]

Some authors refer to the PCR-based method as a 'platinum standard' compared to the so-called 'gold standard' of CSF cultures.[15] However, the equipment needed to perform such assays is not ubiquitous, and still the high price of mPCR methods limits its widespread use in routine practice. Results are usually ready within 2 hours, but the need to transport clinical materials to specialized laboratories equipped with mPCR may increase the time to obtain a test result.[38] Even more, in the so-called California project, the combined testing of patients with encephalitis by conventional and PCR-based methods, Glaser et al. showed that the etiological agent in encephalitis remains unclear in 62% of cases.[49] On the other hand, these methods show significant sensitivity, thus often detecting viral pathogens that survive in a latent state in the CNS, like the herpes family. This in turn creates difficulties in interpreting the results, as these pathogens may also be associated with neuroinfection.

Like any other method, PCR assays have limitations as

well. Thus, research in this field does not stop with the introduction of PCR-based techniques, and the search continues in the direction of new diagnostic methods and approaches in patients with acute CNS infections.

Future perspectives

MALDI-TOF MS is traditionally used for the identification of bacteria and fungi after their isolation on solid growth media. This method can speed up the identification process considerably. Research is currently being performed to evaluate the utilization of the method for direct identification of pathogens from CSF samples. Although some promising results are obtained, there are more tests needed to validate the application of the MALDI-TOF MS in this approach.[51,52]

Immune cells can express a different set of genes in response to environmental stimuli such as infectious agents leading to distinct phenotypes.[53] The advances in molecular biology over the past decades elucidated many of the mechanisms involved in gene regulation and expression. With the current techniques like microarrays and next-generation sequencing (NGS), we have the potential to identify the set of all RNA transcripts, coding, and noncoding, in a given population of cells, known as transcriptomics.[54] Transcriptomics research focuses on identifying which genes are upregulated or downregulated and expression profiles to be made.[54] A study showed that sequencing messenger RNA resulted in a successful differentiation of enteroviral meningitis not only to bacterial meningitis (AUC=0.975) but also herpes meningitis (AUC=0.924) from a whole blood sample.[55] NGS has not yet been found to be superior to the routine methods but the extended coverage of pathogens could be helpful in unidentified neuroinfection.[56] The potential role of these new approaches in the diagnosis of acute CNS infection remains to be further evaluated in near future.

CONCLUSIONS

Establishing the aetiology and choosing the adequate treatment in patients with neuroinfections is a complex process requiring a comprehensive approach following the most likely cause of disease according to age, available risk factors, knowledge of circulating pathogens, and levels of antimicrobial resistance, as well as diagnostic methods available at the laboratory. The implementation of new methods for etiological diagnosis in patients with acute CNS infections is a vital necessity and still needed. Rapid and accurate modern diagnostics would lead to a reduction in hospital stays, reduction of unnecessary hospitalizations, and treatment costs due to the application of inadequate antimicrobial therapy. It would also reduce the side effects in patients and the incidence of emerging local resistance, associated with the broad-spectrum empiric antimicrobial therapy.

Conflicts of Interest

All the authors have no conflicts of interest to declare.

Acknowledgments

Project National University Complex for Biomedical and Applied Research – BBMRI.BG, Contract D01-395/18.12.2020 (Ministry of Education and Science – Medical University – Sofia) within the frame of the Bulgarian National Roadmap for Research Infrastructure.

REFERENCES

1. Brown BL, Fidell A, Ingolia G, et al. Infectious causes and outcomes in patients presenting with cerebral spinal fluid pleocytosis. J Neurovirol 2019; 25(4):448–56.
2. Van de Beek D, Cabellos C, Dzupova O, et al. ESCMID guideline: Diagnosis and treatment of acute bacterial meningitis. Clin Microbiol Infect 2016; 22:S37–62.
3. World Health Organization & Centers for Disease Control and Prevention (U.S.). Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae: WHO manual, 2nd ed. (2011). World Health Organization. Available from: https://apps.who.int/iris/handle/10665/70765
4. Wormser GP, Hasbun R. Bacterial Meningitis. Tunkel Philadelphia: Lippincott Williams & Wilkins; Clin Infect Dis 2002; 34(7):1031.
5. Sandle T. Gram's stain: history and explanation of the fundamental technique of determinative bacteriology. ISTSci Technol J 2004; 54:3–4.
6. Bahr NC, Boulware DR. Methods of rapid diagnosis for the etiology of meningitis in adults. Biomark Med 2014; 8(9):1085–103.
7. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice Guidelines for the Management of Bacterial Meningitis. Clin Infect Dis 2004; 39(9):1267–84.
8. Brouwer MC, Van De Beek D, Heckenberg SGB, et al. Community-acquired Listeria monocytogenes meningitis in adults. Clin Infect Dis 2006; 43(10):1233–8.
9. Costerus JM, Brouwer MC, Bijlsma MW, et al. Community-acquired bacterial meningitis. Curr Opin Infect Dis 2017; 30(1):135–41.
10. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. (e-book). Elsevier Health Sciences; 2019; 1449–576.
11. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. Pediatrics 2001; 108(5):1169–74.
12. Crosswell JM, Nicholson WR, Lennon DR. Rapid sterilisation of cerebrospinal fluid in meningococcal meningitis: Implications for treatment duration. J Paediatr Child Health 2006; 42(4):170–3.
13. Wang YY, Xie BD. Progress on diagnosis of tuberculous meningitis. Methods Mol Biol 2018; 1754:375–86.
14. Cherian A, Thomas SV. Central nervous system tuberculosis. Afr Heal Sci 2011; 11(1):116–27.

15. He T, Kaplan S, Kamboj M, et al. Laboratory diagnosis of central nervous system infection. Curr Infect Dis Rep 2016; 18(11):35.
16. Brouwer MC, Tunkel AR. Epidemiology BD. Diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev 2010; 23(3):467–92.
17. Michael B, Menezes BF, Cunniffe J, et al. Effect of delayed lumbar punctures on the diagnosis of acute bacterial meningitis in adults. Emerg Med J 2010; 27(6):433–8.
18. Brink M, Welinder-Olsson C, Hagberg L. Time window for positive cerebrospinal fluid broad-range bacterial PCR and Streptococcus pneumoniae immunochromatographic test in acute bacterial meningitis. Infect Dis 2015; 47(12):869–77.
19. Etyang AO, Amayo EO, Bhatt SM, et al. Comparison of bedside inoculation of culture media with conventional cerebrospinal fluid culture method in patients with bacterial meningitis. East Afr Med J 2009; 86(10):476–79.
20. Chaudhry SH, Wagstaff D, Gupta A, et al. Enrichment culture of CSF is of limited value in the diagnosis of neonatal meningitis. Eur J Clin Microbiol Infect Dis 2011; 30(7):931–3.
21. Lessing MPA, Bowler ICJ. The value of cerebrospinal fluid enrichment culture in the diagnosis of acute bacterial meningitis. Eur J Clin Microbiol Infect Dis 1996; 15(1):79–82.
22. Shah SS, Hines EM, McGowan KL. Cerebrospinal fluid enrichment broth cultures rarely contribute to the diagnosis of bacterial meningitis. Pediatr Infect Dis J 2012; 31(3):318–20.
23. Calderaro A, Martinelli M, Montecchini S, et al. Higher recovery rate of microorganisms from cerebrospinal fluid samples by the BACTEC culture system in comparison with agar culture. Diagn Microbiol Infect Dis 2016; 84(4):281–86.
24. Ragunathan L, Ramsay M, Borrow R, et al. Clinical features, laboratory findings and management of meningococcal meningitis in England and Wales: report of a 1997 survey. J Infect 2000; 40(1):74–9.
25. Ansari I, Pokhrel Y. Culture proven bacterial meningitis in children: Agents, clinical profile and outcome. Kathmandu Univ Med J 2011; 9(33):36–40.
26. Garg RK. Microbiological diagnosis of tuberculous meningitis: Phenotype to genotype. Indian J Med Res 2019; 150(5):448–57.
27. Garg RK, Rizvi I, Malhotra HS, et al. Management of complex tuberculosis cases: a focus on drug-resistant tuberculous meningitis. Expert Rev Anti Infect Ther 2018; 16(11):813–31.
28. Thwaites GE, Bang ND, Dung NH, et al. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. N Engl J Med 2004; 351(17):1741–51.
29. Miller JM, Binnicker MJ, Campbell S, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis 2018; 67(6):e1–94.
30. Abassi M, Boulware DR, Rhein J. Cryptococcal meningitis: diagnosis and management update. Curr Trop Med Reports 2015; 2(2):90–9.
31. Ginocchio CC, Zhang F, Malhotra A, et al. Development, technical performance, and clinical evaluation of a NucliSens basic kit application for detection of enterovirus RNA in cerebrospinal fluid. J Clin Microbiol 2005; 43(6):2616–23.
32. Nigrovic LE, Kuppermann N, McAdam AJ, et al. Cerebrospinal latex agglutination fails to contribute to the microbiologic diagnosis of pretreated children with meningitis. Pediatr Infect Dis J 2004; 23(8):786–8.
33. Tarafdar K, Rao S, Recco RA, et al. Lack of sensitivity of the latex agglutination test to detect bacterial antigen in the cerebrospinal fluid of patients with culture negative meningitis. Clin Infect Dis 2001; 33(3):406–8.
34. Hayden RT, Frenkel LD. More laboratory testing: greater cost but not necessarily better. Pediatr Infect Dis J 2000; 19(4):290–2.
35. Alam M, Bastakoti B. Therapeutic guidelines: antibiotic version 15. Aust Prescr 2015; 38(4):137.
36. Young N, Thomas M. Meningitis in adults: diagnosis and management. Intern Med J 2018; 48(11):1294–307.
37. Liermann K, Schafler A, Henke A, et al. Evaluation of commercial herpes simplex virus IgG and IgM enzyme immunoassays. J Virol Methods 2014; 199:29–34.
38. Lu H-Z, Bloch KC, Tang Y-W. Molecular techniques in the diagnosis of central nervous system infections. Curr Infect Dis Rep 2002; (4):339–350.
39. Bloch KC, Tang Y-W. Molecular approaches to the diagnosis of meningitis and encephalitis. In: Persing DH, Tenover FC, Hayden RT, et al. editors. Molecular Microbiology: Diagnostic Principles and Practice. 3rd ed. Washington, DC: ASM Press; 2016;287.
40. Ni H, Knight AI, Cartwright K, et al. Polymerase chain reaction for diagnosis of meningococcal meningitis. The Lancet 1992; 340(8833):1432–4.
41. Saravolatz LD, Manzor O, VanderVelde N, et al. Broad range bacterial polymerase chain reaction for early detection of bacterial meningitis. Clin Infect Dis 2003; 36(1):40–5.
42. Corless CE, Guiver M, Borrow R, et al. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using Real-Time PCR. J Clin Microbiol 2001; 39(4):1553–8.
43. Tzanakaki G, Tsopanomichalou M, Kesanopoulos K, et al. Simultaneous single-tube PCR assay for the detection of Neisseria meningitidis, Haemophilus influenzae type b and Streptococcus pneumoniae. Clin Microbiol Infect 2005; 11(5):386–90.
44. Levterova V, Simeonovski I, Kurchatova A, et al. Etiological diagnosis of meningococcal meningitis and serogroup typing of Neisseria meningitidis by real-time PCR in Bulgaria. Probl Inf Paras Dis 2016; 44(2):5–10.
45. Lazarova G, Teneva M, Djeneva H. Meningococcal meningitis: bacteriological and molecular aspects. Trakia J Sci 2014; 12(3):291–5.
46. Iturriza-Gomara M, Megson B, Gray J. Molecular detection and characterization of human enteroviruses directly from clinical samples using RT-PCR and DNA sequencing. J Med Virol 2006; 78(2):243–53.
47. Beld M, Minnaar R, Weel J, et al. Highly sensitive assay for detection of enterovirus in clinical specimens by reverse transcription-PCR with an armored RNA internal control. J Clin Microbiol 2004; 42(7):3059–64.
48. Harvala H, Broberg E, Benschop K, et al. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. Recommendations for enterovirus diagnostics and characterisation within and beyond Europe. J Clin Virol 2018; 101:11–7.
49. Glaser CA, Gilliam S, Schnurr D, et al. In search of encephalitis etiologies: Diagnostic challenges in the California Encephalitis Project, 1998-2000. Clin Infect Dis 2003; 36(6):731–42.
50. Nigrovic LE, Malley R, Macias CG, et al. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. Pediatrics 2008; 122(4):726–30.
51. Bishop B, Geffen Y, Plaut A, et al. The use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for rapid

bacterial identification in patients with smear-positive bacterial meningitis. Clin Microbiol Infect 2018; 24(2):171–74.
52. Segawa S, Sawai S, Murata S, et al. Direct application of MALDI-TOF mass spectrometry to cerebrospinal fluid for rapid pathogen identification in a patient with bacterial meningitis. Clin Chim Acta 2014; 435:59–61.
53. Transcriptomics in Health and Disease. Springer International Publishing; 2014. doi: 10.1007/978-3-319-11985-4
54. Chambers DC, Carew AM, Lukowski SW, et al. Transcriptomics and single-cell RNA-sequencing. Respirology 2019; 24(1):29–36.
55. Bartholomeus E, De Neuter N, Lemay A, et al. Diagnosing enterovirus meningitis via blood transcriptomics: An alternative for lumbar puncture? J Transl Med 2019; 17(1):282.
56. Zhang JZ, Zheng P, Sun HM, et al. Next-generation sequencing combined with routine methods to detect the pathogens of encephalitis/meningitis from a Chinese tertiary pediatric neurology center. J Infect 2019; 78(5):409–21.
57. Kalchev Y. Microbiological and immunological approaches for rapid etiological diagnosis of acute central nervous system infections [PhD Thesis], Plovdiv, Bulgaria: Medical University of Plovdiv; 2020.

Современные методы микробиологической диагностики острых инфекций центральной нервной системы

Йордан Калчев[1,2,3], Мариана Мурджева[1,2,3]

[1] *Кафедра медицинской микробиологии и иммунологии им. „Проф. Д-р Элиссай Янев“, Факультет фармации, Медицинский университет – Пловдив, Пловдив, Болгария*

[2] *Лаборатория микробиологии, УМБАЛ „Св. Георги“, Пловдив, Болгария*

[3] *Научно-исследовательский институт при Медицинском университете – Пловдив, Пловдив, Болгария*

Адрес для корреспонденции: Йордан И. Калчев, Кафедра медицинской микробиологии и иммунологии им. „Проф. Д-р Элиссай Янев“, Факультет фармации, Медицинский университет – Пловдив, Пловдив, Болгария, бул. „Васил Априлов“ № 15A, 4002 Пловдив, Болгария; Email: yordan.kalchev@mu-plovdiv.bg; тел.: +359 898 431919

Дата получения: 27 июля 2021 ♦ **Дата приемки:** 4 октября 2021 ♦ **Дата публикации:** 31 октября 2022

Образец цитирования: Kalchev Y, Murdjeva M. Current methods for microbiological diagnosis of acute central nervous system infections. Folia Med (Plovdiv) 2022;64(5):709-715. doi: 10.3897/folmed.64.e72257.

Резюме

Частота инфекций, поражающих центральную нервную систему, в последние годы увеличилась, что делает нейроинфекции актуальной глобальной проблемой здравоохранения. Центральная нервная система достаточно хорошо защищена от внешней и внутренней среды, хотя и подвержена заражению самыми разнообразными возбудителями. Этиологическое разнообразие ещё более усложняет лечение таких инфекций, поскольку важно правильно определить конкретную причину, чтобы выбрать наиболее подходящую противомикробную терапию. Диагноз ставят на основании не только клинико-эпидемиологических данных, но и результатов клинико-лабораторного и микробиологического исследования ликвора. Цель этой статьи состоит в том, чтобы сделать обзор современных микробиологических методов диагностики острых инфекций центральной нервной системы и помочь медицинским работникам осознать их преимущества и ограничения для надлежащего ведения пациентов.

Ключевые слова

культура ликвора, инфекции ЦНС, прямая микроскопия, реакция латекс-агглютинации, мультиплексная ПЦР, нейроинфекция