Review

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Biomarkers in Medicine

Methods for rapid diagnosis of meningitis etiology in adults

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Infectious meningitis can be caused by viral, bacterial or fungal pathogens. Despite widely available treatments, many types of infectious meningitis are still associated with significant morbidity and mortality. Delay in diagnosis contributes to poor outcomes. Cerebrospinal fluid cultures have been used traditionally but are time intensive and sensitivity is decreased by empiric treatment prior to culture. More rapid techniques such as the cryptococcal lateral flow assay (IMMY), GeneXpert MTB/Rif Ultra (Cepheid) and FilmArray multiplex-PCR (Biofire) are three examples that have drastically changed meningitis diagnostics. This review will discuss a holistic approach to diagnosing bacterial, mycobacterial, viral and fungal meningitis.

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Infectious meningitis is caused by numerous pathogens and may be life threatening [1–3]. The classic symptoms of meningitis are fever, neck stiffness and headache, although these features are not present uniformly [2–4]. Broadly, the most common causes of infectious meningitis are viral, bacterial, mycobacterial and fungal. Parasitic and non-infectious causes of meningitis also occur but are not the focus of this review.

In the USA, meningitis accounts for more than 72,000 hospitalizations with a total expenditure of 1.2 billion dollars annually [5]. Many types of infectious meningitis carry high rates of mortality and long-term complications including neurologic deficits and cognitive impairment [3,6,7]. Mortality due to bacterial meningitis ranges from 10 to 20% in high resource settings and as high as 50% in lower resource settings where bacterial meningitis is estimated as the fourth leading cause of disability [3,6–9]. In comparison to bacterial meningitis, aseptic (typically viral) meningitis typically caries a good prognosis (4.5% mortality rate) [3]. Tuberculous meningitis has a higher mortality rate, up to 50% in HIV-infected subjects and of individuals who survive tuberculosis meningitis, approximately 50% suffer from neurologic disability [6,10]. Like tuberculous meningitis, meningitis due to fungi carries significant mortality. Cryptococcal meningitis carries an acute in-hospital mortality of 30–50% [11]. World-wide, meningitis due to *Cryptococcus* accounts for 15% of AIDS-related deaths [12]. Other forms of fungal meningitis are rarer, but also deadly. Antinori *et al.* reported a case fatality rate of 63.5% due to *Aspergillus* meningitis for immunocompetent and 83% for immunocompromised patients [13]. Meningitis cause by *Coccidioides* has mortality of 90% at 1 year and 100% at 2 years if untreated [14]. CNS infections due to *Histoplasma* have a 39% case fatality rate [15].

It is not always possible to determine the etiology of meningitis. A study by Sulaiman *et al.* only identified the etiology in about 32% of cases, while other studies found similar ranges [16–18]. This is likely related to the lack of sensitivity of CSF cultures for non-bacterial pathogens as well as the underutilization of viral molecular and serologic testing [16]. Of course the etiology in any individual case may be influenced by many factors including test availability, geographic region, host, and many others – the main point is that the exact etiology is not always uncovered. Due to the high mortality and morbidity of many types of meningitis, it is critical to obtain a diagnosis or initiate empiric treatment rapidly as soon as possible [1]. Clinician diagnoses must be informed by historical

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information such as duration of symptoms, travel and country of origin, host immune capabilities, vaccination status, as well as an understanding of the appropriate diagnostic testing based on the probable epidemiology [1]. This review will focus on the diagnosis of bacterial, mycobacterial, fungal meningitis due to their worse prognosis and need for accurate diagnosis as well as the various diagnostic tests used in these conditions. Given viral meningitis typically is generally associated with relatively good outcomes, PCR testing of viral etiologies will not be a major focus of this review.

This is an extensive narrative review. We searched pubmed for 'meningitis and diagnosis', 'epidemiology and meningitis', 'risk factors and meningitis', 'stem cell transplant and meningitis', 'organ transplant and meningitis', 'bacterial meningitis', 'tuberculosis meningitis', 'fungal meningitis', 'histoplasma meningitis', 'coccidioides meningitis', 'cryptococcal meningitis', 'blastomyces meningitis' and 'aspergillus meningitis' to gather data in addition to using material cited in some of the sources found via these searches.

Epidemiology

Although an estimated 16 million cases of bacterial meningitis occurred worldwide in 2013, only 4100 cases per year occur in the USA [2,19]. In Western countries the incidence of bacterial meningitis over the past 10–20 years has declined by approximately 3–4% per year and currently is approximately 0.8 cases per 100,000 per year [20]. The rates of bacterial meningitis are significantly higher in many African countries with an incidence of 10–40 per 100,000 persons per year [20]. The most common etiologies in order of frequency are *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Listeria monocytogenes* [4,20]. Meningitis due to *H. influenzae* type B and *N. meningitidis* has decreased over the past 10–20 years, due to vaccination [3,20,21].

Aseptic meningitis is relatively common with an annual incidence of 7.6 per 100,000 adults in the USA [6]. Enteroviruses, herpes simplex virus, varicella zoster virus and West Nile virus are the most common causes of infectious aseptic meningitis, other causes include cytomegalovirus and human immunodeficiency virus (HIV), among others [18,22,23]. Aseptic meningitis can also be caused by non-infectious etiologies such as adverse reaction to medications, chemotherapy, vaccinations or inflammatory diseases [22,23]. In 30–65% of cases of aseptic meningitis, the etiology is not definitively identified [22].

Tuberculosis (TB) meningitis incidence is not known, but likely occurs in about 1–5% of TB cases worldwide [24]. In 2017 WHO estimated 10 million incident cases of tuberculosis, which would correlate to 100,000–500,000 cases per year of TB meningitis [25].

Worldwide, fungal meningitis causes substantial mortality, particularly among immunocompromised persons [5]. The primary pathogens implicated are *Cryptococcus, Coccidioides*, *Histoplasma*, and *Candida*. In 2014, Rajasingham *et al.* estimated 223,100 incident cases of cryptococcal meningitis with 181,100 annual fatalities [12]. Sub-Saharan Africa accounted for 73% of the 223,1000 cases [12]. Meningitis due to *Cryptococcus neoformans* is most commonly seen in immunocompromised individuals, especially those with HIV [5,26]. However in high-income countries, 30% of cryptococcal meningitis occurs in apparently immunocompetent individuals, particularly if due to *C. gattii* [5]. Meningitis due to *Coccidioides*, *Histoplasmosis* or *Candida* typically occurs as a results of dissemination and so is relatively uncommon in hosts with intact immune systems. Meningeal involvement occurs in 33–50% of disseminated coccidioidomycosis and 10–20% of disseminated histoplasmosis [5].

Regional variation in frequency and etiology of meningitis occurs as well. For instance, the 'meningitis belt' (Senegal to Ethiopia in sub-Saharan Africa) experiences high numbers of bacterial meningitis, prior to the introduction of the conjugate meningococcal vaccine for serotype A [20,21]. In areas with high rates of HIV such as Malawi, South Africa, Uganda, Zambia and Zimbabwe, *Cryptococcus* and TB are the first and second most common causes of meningitis [26]. In Iran, *H. influenza* type B meningitis is relatively common due to a lack of routine vaccination [9]. *Coccidioides* meningitis is a frequent causes of meningitis in Arizona and California whereas *Histoplasma* meningitis is more common in the Mississippi and Ohio river valleys of the United States and much of Latin America [27,28].

Host factors

The likelihood of a particular etiology of meningitis is also affected by the host. In general, bacterial meningitis is more commonly seen in older individuals, possibly due to immune system decline over time [3,4,7]. Adriani *et al.* found that almost half of patients with bacterial meningitis had a predisposing condition and a third had an immunodeficiency (diabetes mellitus, alcoholism, cancer, history of organ transplant, HIV, asplenia, complement deficiency or anatomical defects) [7]. Common bacterial etiologies of *N. meningitidis*, *H. influenzae* and *S. pneumoniae* – all are encapsulated organisms that require protection via B lymphocyte production of immunoglobulins,

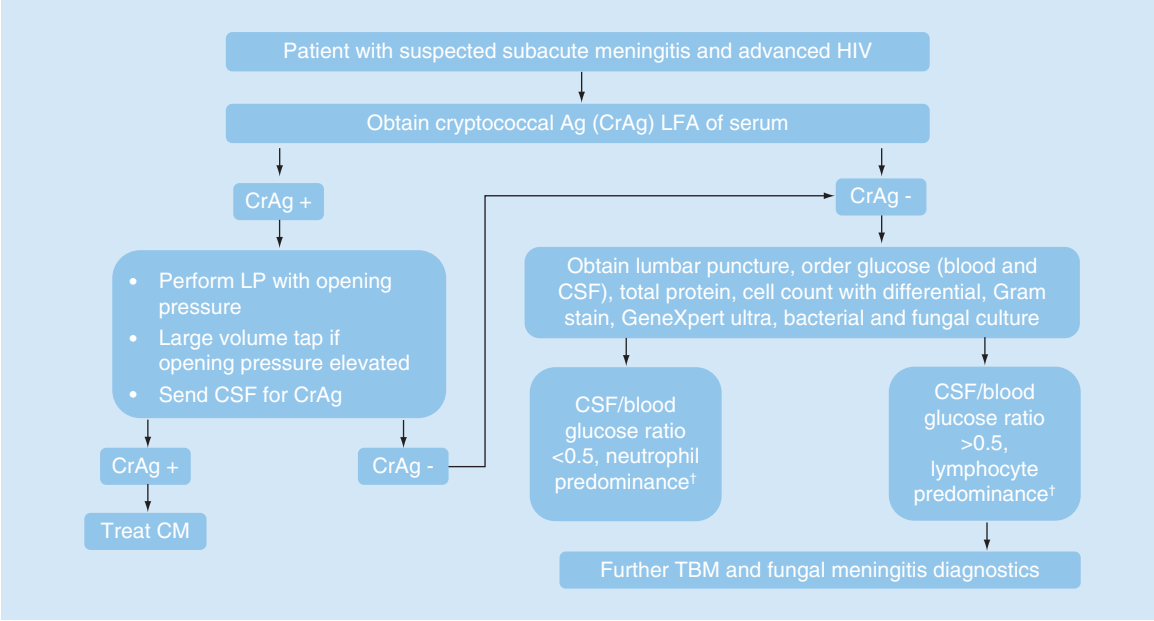

Figure 1. Simplified diagnostic algorithm for diagnosis of subacute meningitis in advanced HIV.
†Most likely bacterial meningitis, particularly *L. monocytogenes*. Institute empiric antibiotics after lumbar puncture (or before if the patient is unstable).
‡Most likely tuberculous meningitis. If acid-fast bacilli smear unremarkable and duration of symptoms correlate strongly consider empiric treatment and/or nucleic acid amplification testing, ideally utilizing a large volume (>5 ml) of centrifuged cerebrospinal fluid. Also consider work up for other fungal meningitis, such as *Aspergillus*, *Blastomyces*, *Coccidioides* and *Histoplasma* in the proper setting.
The Delphi method of consensus was used to construct this figure.
CM: Cryptococcal meningitis; CrAg: Cryptococcal antigen; CSF: Cerebrospinal fluid; LFA: Lateral flow immunochromatographic assay.

immunoglobulin activating the complement cascade, and appropriate splenic function to remove circulating bacteria [3,7,29]. Disruptions, quantity or function of B cells, immunoglobulin, complement and asplenia increase risk of meningitis due to encapsulated organisms [29]. *Candida* meningitis is more common in patients with ventriculoperitoneal shunts, and cochlear implants have been associated with a higher incidence of pneumococcal meningitis [5,7,29]. Head trauma and neurosurgery are associated with higher rates of aerobic Gram-negative bacilli and *Staphylococcus aureus* [2,3].

Fungal (most notably cryptococcal) meningitis is more common in patients with CD4 T-cell dysfunction, most notably persons living with HIV/AIDS [5]. Compared with those without HIV co-infection, persons with TB and HIV are more likely to develop extrapulmonary disease, including tuberculosis meningitis [30]. Finally, HIV-infected individuals are at higher risk than the general population for meningitis due to typical bacterial pathogens such as *S. pneumoniae* as well as less common bacteria such as *Salmonella* and *Listeria* species [7,29]. Figure 1 shows a diagnostic approach to subacute meningitis in persons with advanced HIV, Figure 2 shows a diagnostic approach to subacute meningitis in persons a presumed normal immune system. The figures are based on the literature cited within this review, but also our clinical experience. Of note, Figure 2 refers to the CSF to blood glucose ratio. Glucose is transported across the blood–brain barrier, therefore CSF glucose levels are directly rated to plasma levels [31]. Elevations in the plasma glucose will cause the CSF glucose levels to be elevated but CSF levels are decreased with certain CSF infections. When interpreting CSF glucose levels they should be compared with plasma levels [31]. CSF/blood glucose level is typically low in bacterial and tuberculous meningitis, but can also be seen with other causes as well [3,27,32,33].

TNF-α inhibitors are commonly used for a number of auto-immune conditions and have been linked to numerous infections including histoplasmosis and tuberculosis, including meningitis at a higher rate than the general population [5,34–36]. Moreover, infliximab in particular causes increased incidence of *Listeria* meningitis although other TNF-α inhibitors have been linked as well [34,37,38]. Among solid organ transplant recipients, the

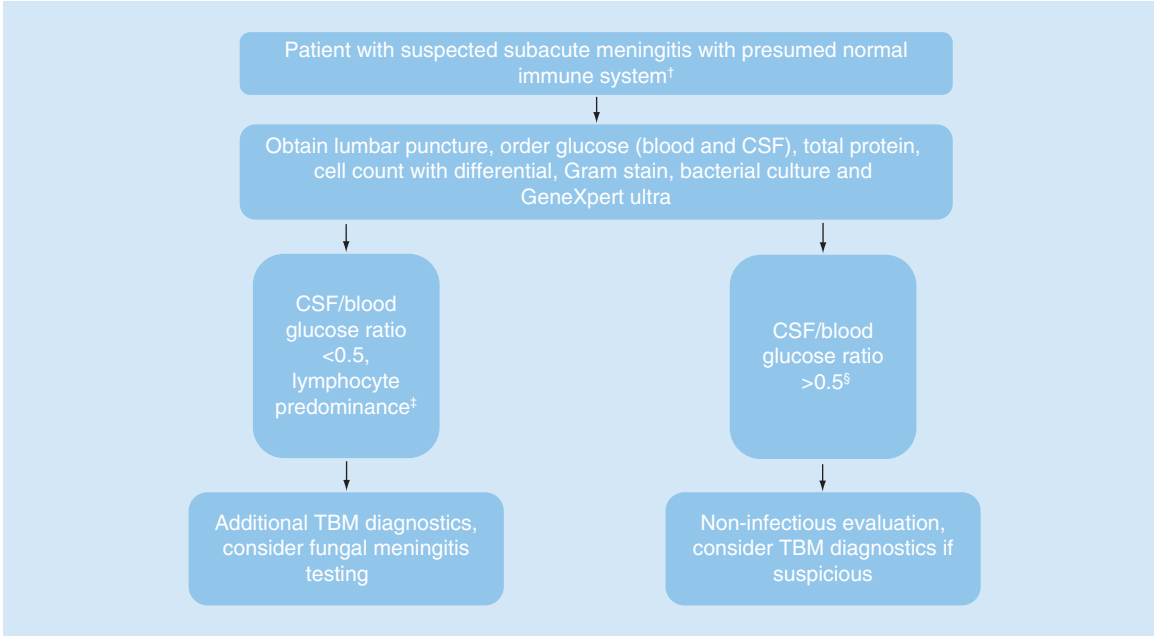

Figure 2. Algorithm for diagnosis of subacute meningitis in patients with presumed normal immune system.
†If appropriate consider rapid HIV test, if HIV-infected, refer to Figure 1 algorithm.
‡Most likely tuberculosis meningitis. If duration of symptoms are compatible, strongly consider empiric treatment and/or NAATs, ideally testing a large volume (>5 ml) of centrifuged cerebrospinal fluid. Consider fungal etiologies as well such as *Aspergillus*, *Blastomyces*, *Coccidioides* and *Histoplasma*.
§Most likely aseptic meningitis, particularly non-infectious etiologies. Although may be tuberculosis meningitis as well. If strong clinical suspicion, consider TB NAATs.
The Delphi method of consensus used to construct this figure.
CrAg: Cryptococcal antigen; CSF: Cerebrospinal fluid; TBM: Tuberculosis meningitis.

annual incidence of bacterial meningitis is 5.4-times higher than the general population, *Listeria* meningitis is of particular concern (110-fold increased risk) [7,39]. Cryptococcal meningitis risk is also high among solid organ transplant recipients and those with cirrhosis [40,41]. Patient who have undergone hematopoietic stem cell transplant (HSCT) are also at increased risk for infectious meningitis, particularly those having undergone allogenic HSCT [42]. *S. pneumoniae*, *N. meningitidis* and *Aspergillus* spp. are of particular concern in the HSCT population [27,42–45].

Duration of symptoms

Meningitis can be broadly categorized as acute (<5 days), subacute (5–30 days), and chronic (>30 days). Bacterial meningitis typically presents in less than 24 h and though viral meningitis may have an acute presentation, generally, the onset of symptoms is not as rapid (median 2 days) nor as severe [3,6,16,46]. However, it is important to note that patients with certain risk factors, such as those with a solid organ transplant, may have a more protracted clinical course compared with the general population [39]. Another exception is *Listeria* meningitis with approximately one third of patients having symptoms for more than four days [1,2]. Subacute or chronic meningitis is typically due to viruses, fungi or mycobacteria (*M. tuberculosis*); 24 weeks of symptoms is most typical of latter two categories [1,16,27,47]. Yet, TB meningitis can occur with as little as 1 week or as long as 1 year of symptoms [33]. Moreover patients with *Histoplasma* meningitis typically have symptoms for at least 2 months and *Blastomyces* meningitis is typically diagnosed after an average of 3 months of symptoms [15,48,49]. Table 1 summarizes typical durations of symptoms by etiology.

General diagnostic tests

Cerebrospinal fluid (CSF) analysis is key to determining the etiology of meningitis [2,46,50]. Nonspecific, standard CSF testing includes white blood cell count with differential, glucose, and protein [1,46]. Initial results return within hours (ideally faster) and can help one differentiate the more likely etiologies. Yet, none of these tests have high specificity and are to be interpreted in the setting of other relevant clinical information. Table 2 shows the typical

Table 1. Comparison of the etiologies of meningitis based on duration of symptoms.

Acute (<5 days)	Subacute (5–30 days)	Chronic (>30 days)
Bacterial†	*Listeria monocytogenes*‡	*Cryptococcus*
Viral	*Cryptococcus*	Tuberculosis
	Tuberculosis	*Histoplasma*
	Histoplasma	*Blastomyces*
	Coccidioides	*Coccidioides*

†Patients with history of SOT can have a more protracted clinical course compared with the general population.
‡One third of patients with *Listeria monocytogenes* have symptoms for more than 4 days.

Table 2. Comparison of typical findings for common cerebrospinal fluid laboratory tests by etiology of meningitis.

Meningitis etiology	Total protein	CSF/blood glucose ratio	Total WBC	Predominant WBC type
Bacterial	+++	+++	+++	Neutrophils
Aseptic (viral)	±	±	±	Lymphocytes†
Tuberculosis	++	+++	++	Lymphocytes
Cryptococcal	++	++	±	Lymphocytes
Coccidioidal	++	+	±	Lymphocytes‡
Blastomyces	++	++	±	Lymphocytes
Histoplasma	++	++	±	Lymphocytes
Aspergillus	++	+	±	Lymphocytes

†May be neutrophil predominant early in the disease course.
‡Eosinophilic predominance occurs less commonly but when seen is highly suggestive.
The number of + signs indicate proportional gradient of increase (total protein or total WBC) or decease (ratio of CSF/blood glucose) of the lab test. To indicate proportionally minimal or no increase/decrease, ± is used. WBC differential indicates the dominant cell type present although other cells generally are present as well.
CSF: Cerebrospinal fluid; WBC: White blood cell.

patterns of basic CSF tests for by etiology.

Testing the CSF for inflammatory markers, such as C-reactive protein (CRP), procalcitonin, lactic acid and ferritin may aid in the differentiation of bacterial from viral meningitis but are not commonly used and have been more frequently studied as blood tests [4,51–53]. It should be noted that CSF lactate, in particular is used in some settings. However, a CSF lactic acid concentration of 4.2 mmol/l or greater has a sensitivity of 96% and specificity of 100% for differentiating bacterial from viral meningitis although elevations also occur in cryptococcal meningitis, TB meningitis and a number of noninfectious conditions such as seizure, ischemia or hemorrhage [52,53]. Other CSF biomarkers to differentiate bacterial from viral meningitis include procalcitonin (cut-off 0.235 pg/ml, sensitivity 96.4%, specificity 80%), ferritin (cut-off 4.6 ng/ml, sensitivity 92.9%, specificity 68%), and CRP (cut-off 1.3 mg/l, sensitivity 92%, specificity 84%) [51]. Finally, CT and magnetic resonance imaging (MRI) of the head can provide additional information in diagnosis of suspected meningitis but in most cases are not helpful in determining the causative agent [27].

Table 3 summarizes the major, specific tests by etiology of meningitis.

Bacterial meningitis except tuberculous meningitis

Culture & Gram stain

CSF culture is considered the gold standard to diagnosis bacterial meningitis, yet is only positive in 70–85% of persons with bacterial meningitis who have not received antimicrobial therapy prior to lumbar puncture [19,51,52]. Final results of the culture are often not available for 48 h or more [19,52,54]. Gram stain is more rapid and has good specificity but sensitivity is poor (10–93% depending on the organism and whether or not antibiotics were given prior to CSF collection) [19,52]. Gram stain is most useful for *S. pneumoniae*.

Latex agglutination

Latex agglutination is relatively inexpensive and rapid (~10 min) [2,6,55–58]. Sensitivity varies by microorganism and manufacturer – 78–100% for *H. influenzae*, 59–100% for *S. pneumoniae*, 69–100% for *S. agalactiae* and 50–93% for *N. meningitis* [4,52]. Kits to detect multiple common bacterial pathogens, have also been developed [8,55]. Latex agglutination may be particularly useful in patients with a negative Gram stain when the LP is performed after

Table 3. Comparison of major commercially available or investigational diagnostic tests by meningitis etiology.

Test	Description	Time to Results	Advantages	Disadvantages	Comm. Avail.	Ref.
Bacterial						
Gram stain	Stain of fluid for bacteria	1 h	Cheap, easy to perform	sensitivity ~90% prior to antibiotics for *S. pneumoniae* meningitis	yes	
Culture	Standard bacterial culture	1–3 days	May grow quickly, easy to perform, adaptable to rapid identification methods	Yield decreased by antibiotic use prior to culture, may be days to results, variable sensitivity	yes	
Procalcitonin, C reactive protein	Serum biomarkers	1 h	Good differentiation between bacterial and aseptic meningitis	Cost, lab requirements, no studies on TBM or CM	yes	
Lactate	Biomarker measure in CSF	<5–60 min	Rapid, sensitive and specific if obtained prior to antibiotics	Not very sensitive if measured after antibiotics are given	yes	[170]
16s rRNA PCR	PCR detection of 16s ribosomal RNA to elicit specific pathogens	Hours	Rapid, more sensitive than culture, very specific	Extremely costly, requires lab expertise and infrastructure	Yes	
Nucleic acid amplification tests	Specific RT-PCR and LAMP assays have been tested for particular pathogens	1–2 h	Rapid, specific, potentially quite sensitive	Cost, lab infrastructure, lack of large studies	In some cases	
Rapid diagnostic tests	Rapid, usually card or dipstick-based tests for specific etiologies	<15 min	Rapid, cheap, easy to use, no significant lab infrastructure necessary	Variable specificity, sensitivity	yes	
MALDI-TOF MS	Mass spectrometry identification based on weight	1–2 h	Rapid, relatively inexpensive	Requires significant laboratory infrastructure, not widely used on CSF at this time	Yes, blood only	
Mycobacteria tuberculosis						
Ziehl–Neelsen stain	Staining for acid-fast bacilli	1 h	Cheap	Very insensitive, minimal utility. Extremely technician dependent	yes	
LJ culture	Traditional culture, solid media	3–5 weeks	Reliable, somewhat sensitive	Very slow growth, still many false negatives, costly, labor intensive	yes	
MGIT culture	Liquid-based culture	1–2 weeks	As sensitive and quicker than LJ culture	~2 weeks to growth, costly	yes	
Adenosine deaminase activity (ADA)	Detectable enzyme released by during T cell activation	<1 h	Rapid, low cost	Variable sensitivity and specificity, lab infrastructure	yes	
Interferon gamma release assay (IGRA)	IFN-g secretion by host memory T cells on exposure to TB antigens	24–36 hours	Good sensitivity	Labor intensive, costly, high numbers of indeterminate results, variable studied cut-points, rely on T-cell function	yes	
PCR	Traditional PCR	Hours	Fast, nearly as sensitive as culture, specific	Cost, lab expertise, lab apparatus, inadequate sensitivity	yes	
LAMP	DNA amplification different from typical PCR, detection by color change		Less lab expertise and infrastructure required than PCR, isothermal	No data on performance	no	
GeneXpert	Cartridge-based PCR	2.5 h	Quick, similar sensitivity to culture, specific, ease of use	Cost, requires significant infrastructure, limited shelf life on cartridges	Yes	
GeneXpert ultra	Cartridge-based PCR	<2 h	Rapid, improved performance versus any commercially available test, ease of use	Cost, lab infrastructure, still not adequate negative predictive value to 'rule-out' TB meningitis	Yes	
Cryptococcus						
CrAg lateral flow assay	Rapid dipstick test detects cryptococcal antigen	10 min	Very sensitive, specific, cheap, does not require significant lab capacity	Cannot differentiate active from past infection	yes	
Culture	Traditional culture	3–14 days	Very accurate, can decide active from past infection	Slow, labor intensive	yes	
CrAg latex agglutination or ELISA	Lab-based detection of cryptococcal antigen	1 day	Sensitive and specific. Requires lab infrastructure	Costly, lab capacity requirement, + result lingers for years	yes	

Test is meant to describe test category, not each specific commercial test. The description notes how the test works in principle. 'Pro' and 'Con' refer to positive and negative aspects of each tests performance and utility. Assays dealing with *M. tuberculosis* cells require increased biosafety apparatus.
AFB: Acid-fast bacilli; CF: Complement fixation; Comm. Avail.: Commercially available; CrAg: Cryptococcal antigen; CSF: Cerebrospinal fluid; ID: Immunodiffusion; IFNg: Interferon gamma; LAMP: Loop mediated isothermal amplification; LJ: Lowenstein Jensen; MALDI TOF MS: Matrix-assisted laser desorption/ionization time of flight mass spectrometry; MGIT: Mycobacterial growth indicator tube; NAAT: Nucleic acid amplification test; PCR: Polymerase chain reaction; rRNA: Ribosomal ribonucleic acid.

Table 3. Comparison of major commercially available or investigational diagnostic tests by meningitis etiology (cont.).

Test	Description	Time to Results	Advantages	Disadvantages	Comm. Avail.	Ref.
India ink	Staining for *C neoformans capsule*	15 min	Inexpensive, easy to perform	85% sensitive; Technician dependent	yes	
Histoplasma						
Culture	Traditional culture	Weeks	Very accurate, widely available	Slow, low yield	yes	
Antibody/ antigen testing	Immunodiffusion, complement fixation, EIA	Hours	Inexpensive	Can be negative early in infections, can cross react with other fungi	yes	
Coddidioides						
Culture	Traditional culture	Weeks	Very accurate, widely available	Slow, low yield	yes	
Wet mount	Direct visualization of organism	<1 h	Inexpensive, easy to perform	Very low yield, requires experienced lab personnel	yes	
Antibody/antigen testing	CF, ID, EIA	Hours–days	Inexpensive	Cannot 'rule out', cross reactivity with other fungi	yes	
Blastomyces						
Culture	Traditional culture	Weeks	Very accurate, widely available	Slow, low yield	Yes	
Antigen testing	EIA	Days		Minimal performance data, reference lab only	No	
Aspergillus						
Culture	Traditional culture	Weeks	Widely available	Slow, low yield, requires multiple samples	Yes	
Galactomannan	Detection of Ag to cell wall component	Hours	Widely available	Cross reacts other species and medications	Yes	
Aseptic (viral)						
16s rRNA amplification	PCR detection of 16s ribosomal RNA to elicit specific pathogens	Days	Rapid, very specific	Extremely costly, requires lab expertise and infrastructure	Yes	
NAATs	Specific PCR and RT-PCR assays for certain pathogens	1–6 h	Rapid, specific	Cost, lab infrastructure and expertise	In some cases	
Syndromic or pan-pathogenic						
BioFire film array	PCR panel that detects 14 h pathogens	1 h	Rapid, specific	Limited to pathogens in the panel costly, lab infrastructure	Yes	
Next-generation sequencing	Nucleic acid detection of any pathogen	Days	Specific, unclear clinical role	Only available at one center, costly, lab infrastructure	Yes	

Test is meant to describe test category, not each specific commercial test. The description notes how the test works in principle. 'Pro' and 'Con' refer to positive and negative aspects of each tests performance and utility. Assays dealing with *M. tuberculosis* cells require increased biosafety apparatus.
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antibiotic administration or among bacteria with fastidious culture growth [6,55,59]. Yet, overall use is limited by inadequate negative predictive value (e.g., inability to rule out bacterial meningitis), and so latex agglutination is only sporadically used.

Limulus lysate assay to detect endotoxin

The limulus lysate assay utilizes lysate prepared from the amebocyte of the horseshoe crab, *Limulus polyphemus*, to identify endotoxins [52,60,61]. The test was originally used for detection of lipopolysaccharide endotoxin in blood, urine and pharmaceutical products, but has been adapted to CSF [60,61]. Results are typically available in 20–30 min and a positive test suggests Gram-negative bacterial meningitis [52,60]. As this assay detects only endotoxin, its inability to detect Gram-positive bacteria is a major limitation [52,60]. In suspected bacterial meningitis without an etiology determined by culture, this assay can help identify if etiology is from a Gram-negative organism.

Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR) testing can provide rapid and accurate diagnoses for bacterial meningitis [2,62]. Depending on the method used, results can be available in as little

Table 4. Comparison of target genes for commercially available bacterial meningitis PCR assays.

Species	Multipex LightMix real-time PCR assay	Speed-oligo bacterial meningitis test	Fast track bacterial meningitis kit
Streptococcus pneumoniae	*lytA*	*lytA*	*lytA*
Neisseria meningitidis	*ctrA*	*ctrA*	*ctrA*
Haemophilus influenzae	*hpd*	*bexA*	*ompP2*
Listeria monocytogenes	*hlyA*		
Streptococcus agalactiae	*cfb*		

as 15 min, and no longer than 3 h for a single sample [63–68]. Of course, if the PCR test is not performed on site, shipping time increases the time to results significantly.

Further, commercial NAATs have been developed to detect multiple bacterial pathogens (some include viral and fungal pathogens as well) and while sensitivities and specificities are often >90% for each pathogen, test performance varies by pathogen and assay [4,52,62,63,65–70]. Table 4 summarizes three of the commercially available PCR assays for bacterial meningitis that detect multiple pathogens and their gene targets. For example, the BioFire Film Array meningitis/encephalitis panel (FilmArray ME) is a PCR panel that rapidly (1 h) detects 14 pathogens including six bacteria, seven bacteria and one fungus directly from CSF [19,71]. Leber *et al.* found that the FilmArray ME panel had an overall percentage of agreement with comparative testing of 99.8% and a specificity of 99.2% [19]. However, this panel does not perform equally for all pathogens and is particularly weak for *Cryptococcus* and herpes simplex 1 and 2 compared with standard diagnostics for these conditions [19,71]. Further, the FilmArray ME panel and all NAATs only detect the pathogens they were designed to target and so will miss pathogens that they are not designed to target.

Further limitations of NAATs include cost, infrastructure requirements, easy contamination of samples, and the need for additional laboratory personnel training [63,66–68,70]. However, NAATs may be particularly useful for the diagnosis of bacterial meningitis when patients have received antibiotics prior to LP. Numerous NAATs technologies are currently being utilized in high-income countries [2]. Future developments in point of care testing (e.g., cartridge based) may facilitate more wide use of NAATs in smaller hospitals and low-resource settings with less training [70].

Loop-mediated isothermal amplification (LAMP) is a NAAT that can be used to detect bacterial DNA [72,73]. LAMP uses DNA primers and polymerases that bind to target DNA which is subsequently amplified [74]. Importantly, LAMP is meant to be isothermal and so require much less laboratory infrastructure. Further, its positivity can be interpreted by the naked eye. Compared with PCR, LAMP is less expensive and requires less extensive laboratory resources [73]. LAMP assays have been shown to have a sensitivity of up to 90% and specificity of up to 100% with capability of detecting pathogens in 20–60 min and at >100-fold lower detection limits than traditional PCR [72,73,75]. However, until recently multiplexing via LAMP has not been possible, although a recent LAMP assay was developed to detect three bacterial pathogens via LAMP integration into a chip [73,75]. This method has potential applicability as a point of care diagnostic test for bacterial meningitis in low-resource settings.

Recently, new molecular techniques have been developed that can detect a pathogen's nucleic acid by harnessing clustered regularly interspaced short palindromic repeats (CRISPER) and CRISPR associated sequences (Cas) related technology [76–79]. Gootenberg *et al.* utilized the Cas13a enzyme to develop a platform called Specific High-Sensitivity Enzymatic Reporter Unlocking (SHERLOCK) that can rapidly detect selected Gram-negative pathogens and distinguish them from other bacteria [77,78,80,81]. Whether these technologies have clinical utility is uncertain – to our knowledge they have not been tested on CSF.

Next-generation sequencing (NGS) has the capability to detect a wide variety of pathogens. Two recent studies used NGS to detect bacterial (*S. pneumoniae, H. influenzae, Enterococcus faecium* and *S. aureus*) and viral pathogens in CSF [82,83]. These reports, combined with literature regarding CSF NGS in subacute meningitis lend themselves to optimism regarding a potential role for NGS in pathogen detection in difficult to diagnose meningitis, yet, this field is young and role of NGS in meningitis is unclear [84,85].

Matrix-assisted laser desorption ionization time of flight mass spectrometry

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) is routinely being used in many centers in high-income countries to quickly identify bacteria from blood cultures and recently has been adapted for use on CSF [2,86,87]. MALDI-TOF MS identifies organisms by comparing the mass spectrum of the test isolate to the database of reference spectra [86,88]. Hartmeyer *et al.* described using MALDI-TOF MS to identify *S.*

pneumoniae in CSF within 1.5 h from receiving the sample [86,89]. The CSF culture subsequently grew *S. pneumoniae*, confirming the diagnosis [86]. Similarly, Segawa *et al.* described rapid identification of *Klebsiella pneumoniae* from CSF by MALDI-TOF MS [89]. The cost of identifying bacteria via MALD-TOF MS is approximately 17–32% of traditional identification methods although the up-front cost of purchasing the equipment is significant and performance varies by pathogen [87]. MALDI-TOF MS is limited by the amount of organism present and can be improved by sample centrifugation [87]. MALDI-TOF MS use for meningitis is experimental at this point but may hold some promise for future use.

Tuberculosis meningitis

Multiple clinical diagnostic criteria have been developed to identify patients likely to have TBM, however none of these have proven broadly generalizable, or adequate as the sole tool for diagnosis of TBM [33,90].

Culture & AFB smear

CSF acid-fast bacillus (AFB) smear microscopy and culture are the typical methods of diagnosis of TB meningitis (TBM) but neither is adequate [91–96]. AFB smear is a microscopic technique that uses Ziehl–Neelsen stain to visualize organisms under direct microscopy [95]. AFB smear is rapid, affordable and widely available but has a low sensitivity (10–20%) [27,30,91,93,97,98]. AFB smear can be improved by examination of at least 6 ml of CSF, examining the sample for at least 30 min or modification of the Ziehl–Neelsen stain using cytospin slides with Triton processing [30,95,97,99]. Lowenstein–Jensen (LJ) and mycobacterial growth inhibitor tube (MGIT) are positive in 45–60% of TBM cases but results are too slow for clinical decision making (6 weeks for LJ, 2–4 weeks for MGIT) [27,91,93,96,98]. Despite sensitivity limitations and biosafety requirements, culture is essential for drug susceptibility testing [98]. In low-resource settings, biosafety requirements are generally only available at national reference laboratories, causing diagnostic delay [98].

Host-derived tests

CSF adenosine deaminase has shown sensitivities ranging from 50 to 100% and specificity from 63 to 99%, further, adenosine deaminase has difficulty distinguishing TBM from bacterial meningitis [47,97,100–102]. CSF adenosine deaminase cannot be used alone to rule out TBM [97,102,103]. Variable performance, cost and infrastructure requirements have limited use worldwide [103]. TB IFN-γ release assays measure host T lymphocyte release of IFN-γ when stimulated by *M. tuberculosis* specific antigens to diagnose latent and active tuberculosis [91,92]. CSF IFN-γ release assays show sensitivity of 75–92% and specificity of 90–100% [92,94,99]. IFN-γ release assays are limited by frequent indeterminate results in those co-infected with HIV, high CSF volume requirement (>2 ml), laboratory infrastructure and lab personnel training [91,92,94,97,99,103].

Delta-like 1 ligand, fetuin and vitamin D binding protein are other immune biomarkers that have been considered for TBM, yet have shown limited utility in an African HIV-infected population [104]. Heat shock proteins found at higher levels in CSF in patients with TBM compared with non-TBM patients and so have been theorized to be a biomarker for TBM – their role as a potential diagnostic test is not clear [100]. Though no immune markers have proven to be adequate diagnostic tests for TBM thus far, given the inadequate of performance of any one test to detect TB bacilli to date in CSF, they may play a role as adjunctive tests in the future [103].

Nucleic acid amplification tests

GeneXpert MTB/Rif (Xpert) is an automated, rapid, cartridge-based PCR assay designed to detect *M. tuberculosis* DNA (and rifampin resistance) in clinical samples [26,27,30,47,91,94,105,106]. Xpert has shown variable performance on CSF in different study populations, but in general has sensitivities similar to culture (50–60%) with a much more rapid turnaround (~2 h) [26,27,30,47,94,105,106]. Specificity has generally been excellent, those studies with possible false-positive results may have been required to label them as such due to the inadequacy of TBM reference standards – the likelihood of false detection of *M. tuberculosis* DNA in the CSF in the setting of chronic meningitis in those without an alternative diagnosis is quite low. The sensitivity of Xpert is higher in HIV-infected individuals, and when higher volumes (at least 5 ml) of CSF are centrifuged [94,105,107]. This cartridge-based assay is easy to use, carries low risk for sample cross-contamination, and does not require a specific biological safety environment as does TB culture [106,108]. However, sensitivity with Xpert is still inadequate, rifampin resistance performance unreliable and despite lower prices in low-resource settings, the prices of the modules and the cartridges are still relatively

high. Inadequate negative predictive value makes Xpert unsuitable as a single 'rule-out' test for TBM [105,106,109]. Still, this technology has widely adopted in TB endemic countries [94,97].

Subsequently GeneXpert MTB/Rif Ultra (Xpert Ultra) was developed as a new assay using the Xpert platform to improve sensitivity and performance of rifampin resistance detection and deliver semiquantitative results in 90 min [10,103,110,111]. Xpert showed sensitivity of 95% for microbiologically proven, definitive TBM versus 45% for Xpert or culture and 70% for probable or definite TBM versus 43% for Xpert or culture [10]. Xpert Ultra has 93–99% negative predictive value versus (90%) for Xpert in TBM depending on the standard used [10,111]. Since March 2017, the WHO has recommended the use of Xpert Ultra as a replacement for Xpert, however addition studies are needed to confirm these findings [110].

Amplicor MTB (Cobas, Amplicor) and Amplified *M. tuberculosis* Direct Test (MTD) are among the commercially available PCR platforms [112–116]. Amplicor PCR has a sensitivity of ~40% and specificity of ~90–100% for TBM, similar to Xpert [112,114]. Yet, false-positive CSF results with Amplicor have been documents in persons with herpes encephalitis, staphylococcal spondylitis and cerebral symptoms secondary to drug interactions [113]. Further, these platforms are generally only located in reference laboratories, require trained laboratory personal, are not fully contained, and are expensive limiting their broad utility [105,115]. The Gen-Probe amplified *M. tuberculosis* Direct test (AMTD) is a transcription-medicated amplification procedure that detects *M. tuberculosis* complex rNA directly from specimens [117]. While data on extrapulmonary specimens in general in promising, studies on its use on CSF specimens are limited [117,118].

LAMP has also been used to diagnose of TBM [94,98,119]. LAMP amplifies DNA in single tube at a constant temperature in only 60 min, potentially making it more suitable for resource-limited settings [94,98,119]. LAMP based assays have been compared with conventional PCR and with sensitivities of 88–96% compared with 53% with conventional in-house PCR [94,99,119]. Despite its advantages, further study is required before widespread adoption of LAMP. Lastly, as recently noted by Michael Wilson and colleagues, NGS may play a role in detection of *M. tuberculosis* in CSF [85].

Other TB meningitis testing

Detection of TB lipoarabinomannan (LAM) in the CSF has been explored as a potential diagnostic technique for TBM [91,94,98,100,120]. Patel *et al.* tested CSF for LAM antigen and found a sensitivity of 64% and specificity of 69% for TBM [120]. Cox, *et al.* found 75% sensitivity and 80% specificity for definite TBM using large volume postmortem CSF samples, tested with the LAM lateral flow assay (LFA, Alere, MA, USA) [121] Others found zero positive results among 67 samples (12 with definite TBM) tested with the same LAM LFA on CSF obtained from LP [122]. Immunocytochemical staining is used to identify antigens within the cytoplasm of macrophages and one study found a sensitivity of 73% and specificity of up to 100% for TBM in CSF [123]. Although immunocytochemical staining has been developed into an assay for wider use, it still requires expertise and so its role TBM diagnosis is uncertain [123].

Diagnostic techniques for multiple types fungal meningitis

1,3-Beta-D-glucan

1,3-Beta-D-glucan (BDG) measurement in CSF has been used to detect a variety of fungi including all fungi discussed below, yet specificity for any individual fungi is inadequate [14,124,125]. Although it was previously thought that *Cryptococcus* did not release sufficient BDG for detection, this was disproven by Rhein and colleagues who found that among HIV-infected patients with cryptococcal meningitis, BDG showed a sensitivity of 89% and specificity of 85% compared with cryptococcal antigen detection with higher BDG levels being associated with mortality [11,125,126]. There remains a FDA-required black-box label incorrectly stating that *Cryptococcus* does not produce BDG. CSF BDG has also been studied for *Histoplasma* meningitis with a sensitivity of 53% and specificity of 87% [124]. Thus, while BDG may have utility in steering clinicians towards fungal meningitis – BDG is not able to reliably diagnose any particular fungal pathogen. A positive BDG result should prompt further focused fungal diagnostics and strong consideration of antifungal therapy.

Next-generation sequencing

NGS is an emerging diagnostic testing platform for subacute and chronic meningitis, including fungal meningitis. Initial results are promising, however further study is required to fully elucidate the role of NGS in the diagnosis of fungal meningitis [84,85,127].

Histoplasma meningitis

CSF culture is the standard for diagnosis of *Histoplasma* meningitis but is only positive in 19–65% of cases and growth can take up to 4 weeks [15,27,124,128–131]. Culture can be improved by using at least three samples of at least 10 ml of CSF – impossible in most cases [15,128,131,132].

Antibodies/antigen

Immunodiffusion, complement fixation and enzyme immunoassay (EIA) can be used to detect *Histoplasma* antibodies in CSF [15,28,124,128–130,133,134]. Moreover, a new IgM and IgG EIA showed sensitivity of 82% (vs 51% for immunodiffusion or complement fixation) and specificity of 93% to diagnose *Histoplasma* meningitis [128]. Yet, antibodies are typically not able to be detected early in infection, and may persist for years after infection [15,135,136]. False-positive CSF results can occur in patients with high levels of serum antibodies due to passive diffusion when blood–brain barrier is impaired due to inflammation or due to cross-reaction with other fungi [129,137]. Antibody response may be impaired in immunosuppressed individuals [132].

Histoplasma antigen can be detected in CSF via EIA [15,124,128–130,134]. Multiple generations of EIA have been designed with the most recent allowing quantification [136]. Antigen sensitivity is as high as 85% when ethylene diamine tetraacetic acid (EDTA) pre-treatment is used [128]. As with antibody testing, false-positive results can occur due to cross-reactivity with other fungi, passive diffusion across the blood–brain barrier or traumatic LP [27,128,129,132,136]. Testing for the organism in non-CNS specimens can also be useful when *Histoplasma* meningitis is secondary to disseminated histoplasmosis [15,27,132,133]. Finally, combined, CSF antigen and antibody testing have shown synergistic performance in one study [128].

Coccidioides meningitis

Wet mount and cytospin preparation can be used to identify *Coccidioides* spherules in CSF to confirm diagnosis; however, the spherules are seen in less than 10% of cases [138–140]. CSF culture is only positive in 15–30% of the cases of coccidioidal meningitis although using large volumes of CSF can improve yield [27,138,139,141,142]. Eosinophilic predominance in CSF can be an indicator of coccidioidal meningitis, but is not specific [139,142,143]. Meningeal biopsy can be used but is invasive and so is rarely used.

CSF anti-coccidioidal antibodies may be detected via immunodiffusion, complement fixation or EIA [27,138–140,142,144]. Complement fixation has a sensitivity of 59–94% while EIA has sensitivity as high as 85%, both have excellent specificities [27,138]. Latex agglutination is less sensitive with more false positives [27,145]. Antibodies less helpful due to limited production early in infection or in immunocompromised persons, and prior (or current) coccidioidomycosis exposure may lead to antibody leakage from the serum into the CSF leading to false-positive CSF testing [14,27,138,139,141,142,144,145]. Cross-reaction with may occur with endemic fungi or *Cryptococcus* [137].

Antigen detection via EIA exhibits 89–93% sensitivity and 100% specificity and as opposed to antibody detection, may have a role in differentiating immune reconstitution inflammatory syndrome (IRIS) from relapse [138,141]. Limitations include cross-reactivity with other endemic fungi, such as histoplasma [138,141]. *Coccidioides* antigen detection is currently available only via reference laboratory, limiting its speed [138]. PCR assays for *Coccidioides* have been used but are not routinely available [27]. Testing by more than one modality and specimen type (e.g., urine, serum, CSF, etc) may increase diagnostic yield [138,145]. For example, one study showed combined antigen and antibody testing with 98% sensitivity and specificity [138].

Cryptococcal meningitis

The most accurate method to diagnose cryptococcal meningitis is cryptococcal antigen detection although culture is important in some situations [11]. India ink staining of the CSF for direct identification of *Cryptococcus* is a readily available technique however it can be lead to missed diagnosis, sensitivity is as low as 42% when fungal burden is <1000 colony-forming units/ml, and the best case is 85% sensitivity [11,27,146–148]. Fungal culture is considered the standard for diagnosis of cryptococcal meningitis, growth can take up to 10 days and false-negative results can occur with a low fungal burden although using higher CSF volume (100 vs 10 µl) can improve sensitivity [11,147]. Despite the limitations of quantitative fungal culture, quantitative culture remains highly clinically useful for monitoring response to treatment and critical for differentiating relapse of cryptococcal meningitis from IRIS, where cryptococcal antigen detection falls short [26]. The FilmArray ME PCR panel has the capability of detecting *Cryptococcus* in CSF but is inadequate compared with antigen testing and it's exact role in the diagnosis of cryptococcal meningitis is not well defined [11,149,150]. FilmArray ME has distinct diagnostic

utility for distinguishing probable culture-positive relapse from culture-negative paradoxical IRIS. NGS can detect *Cryptococcus* in CSF samples, but its role is not yet clear [127].

Cryptococcus capsular polysaccharide antigen

Detection of *Cryptococcus* capsular polysaccharide antigen (CrAg) in CSF, serum, plasma or whole blood is key to rapid diagnosis of cryptococcal meningitis [11]. This test was previously accomplished via latex agglutination or EIA; however in 2011 a CrAg lateral flow assay (LFA) was developed [11,146,148,151–153]. CrAg LFA is a point of care test with results available in 10 min, stability at room temperature (crucial for use in many locations where the infrastructure for cold-chain storage of tests is not practical), and relatively low cost at US$2 per test [11,26,148,151,152]. CrAg LFA in CSF has sensitivity and specificity as high as 99% with whole blood, serum and plasma, being nearly as accurate for diagnosis of meningitis [11,27,40,146–148,152,154,155]. This combination of accuracy, rapidity, cost and heat stability has led to widespread adoption of the CrAg LFA. Yet, lower sensitivity (91%) has been noted with high fungal burdens due to the pro-zone or hook effect (high cryptococcal load interfering with antigen–antibody complex of the assay resulting in a false negative) – this issue resolves with dilution (sensitivity 100%) [11,146,156]. In addition, false-positive results have been reported in lower incidence settings at low titers [157]. Importantly, although a quantitative titer can provide prognostic information, CrAg does not decay at a reliable rate from the CSF (can remain for years) and so cannot be used to monitor treatment response or differentiate IRIS from fungal relapse [11,27,146,152].

A recent study identified a group of HIV infected patients with symptomatic meningitis, positive serum CrAg, but negative CSF testing [158]. Thus, blood CrAg should be obtained in any immunocompromised patient in which Cryptococcal meningitis is suspected as very low antigen in CSF may be too low to detect while higher levels are present in the blood while true cryptococcal meningitis exists – this is pathophysiologically plausible given that *Cryptococcus* first enters the lungs, then disseminates and eventually seeds the CNS [158]. Because serum CrAg can be detected in blood 3 weeks prior to the onset of symptoms of meningitis (with typically a median of another 2 weeks until meningitis diagnosis), targeted serum CrAg screening, can allow detection and treatment of early, subclinical cryptococcosis and prevention of meningitis [11,151,158,159]. Thus, the World Health Organization (WHO) and many national HIV guidelines strongly recommend CrAg screening in HIV patients with a CD4 <100 cells/μl and to consider CrAg screening among CD4 counts 100–200 cells/μl [146,151,158,159].

Blastomyces meningitis

Diagnosis of *Blastomyces* meningitis is difficult and often delayed [160,161]. CSF culture is the standard diagnostic test and takes at least 5 days, but may take up to 4 weeks and is only 64% sensitive [27,49,160,162–165]. CSF culture is more sensitive using fourth ventricle CSF, but this is not practical in most cases [160,162–164]. EIA detection of *Blastomyces* antigen in CSF has been reported but diagnostic performance is unclear [160,161]. Given that *Blastomyces* antigen testing in other body fluids frequently cross reacts with other fungi (particularly *Histoplasma*), cross-reaction in the CSF would be a concern as well [27,160,161]. Serological assays are commercially available for *Blastomyces* and can be used on CSF; however, they are of limited utility for diagnosis of meningitis due to low sensitivity and specificity [27,48,163]. Tissue biopsy or diagnosis of blastomycosis outside of the CNS are other diagnostic options [49,160,162,163,166].

Aspergillus meningitis

Aspergillus meningitis diagnosis is also difficult and in many cases is not obtained until postmortem examination [13,45,167]. In a study by Antinori, *et al.*, only 56% of patients were diagnosed prior to death [13]. CSF fungal culture is only 31% sensitive among all hosts, and 18% in immunocompromised hosts [13,43,44,168,169]. Multiple, large volume CSF cultures may improve sensitivity [13,167]. Galactomannn antigen is a cell wall polysaccharide released by *Aspergillus* that can be detected in body fluids including CSF via ELISA, radioimmunoassay, latex agglutination or western blot [44]. CSF galactomannan sensitivity is 70–90% and specificity 70–100% [13,27,44,169]. As opposed to cryptococcal antigen, CSF *Aspergillus* galactomannan antigen declines with treatment and so may be used to monitor treatment response [167,169]. Cross-reactivity to piperacillin–tazobactam has been reported [44]. *Aspergillus* antibody detection in CSF is possible but performance is unreliable and although PCR has detected *Aspergillus* in CSF, diagnostic performance is unclear [45,167]. Often the diagnosis of *Aspergillus* meningitis occurs only after aspergillosis is diagnosed elsewhere [27].

Viral meningitis

Viral meningitis has significantly less mortality compared with other types of meningitis, and treatment is limited to supportive measures in most cases [23]. NAATs are often used for diagnosis of specific pathogens. Some argue that these tests are underutilized, as positive results may allow rapid discontinuation of empiric antibiotics and decrease length of hospitalization [6,23,52]. Others test only for those viral etiologies with the potential for treatment (e.g., herpes viruses) or those with the potential for more poor outcomes [23].

Barriers to adoption

The largest barrier to adoption of many of the technologies discussed remains cost, this is particularly true for newer molecular techniques such as those utilizing CRISPR or NGS. Excellent performance, minimal lab equipment and personnel training requirements, heat stability and ease of use are all crucial for adoption of new technologies as well. The CrAg LFA remains the standard to which other diagnostic tests for meningitis must strive. Performance and cost are optimal. The CrAg LFA test is easy to use and can be performed at the bedside. CrAg LFA does not require extensive training, a steady electrical supply or cold-chain distribution. For CSF diagnostic tests to be truly accessible and useful worldwide, all of these attributes must be met. Thus, noncryptococcal meningitis diagnostic tests continue to require improvements. While syndromic PCR panels are attractive in terms of requiring smaller volumes of CSF to detect multiple pathogens (e.g., Biofire FilmArray ME), their limitations must be considered. First, these are only able to detect pathogens that they are designed to detect, which is generally appropriate based on high-income country epidemiology of meningitis, yet multiplex panels may miss unexpected pathogens, thus a thorough history and expert physician diagnostic acumen is still required. This seems obvious but the lack of TB in the panel means this panel is less useful in most low and middle resource countries. Further, to truly use the panel correctly, one must understand its limitations and supplement the panel as needed (e.g., the FilmArray ME panel does not eliminate the need for CrAg testing).

Conclusion

Despite the existence of numerous diagnostic techniques, determining the etiology of infectious meningitis remains difficult and cumbersome in many cases. Delay in diagnosis is a significant contributor to mortality in bacterial, tuberculosis and fungal meningitis. When evaluating a patient for meningitis, physicians must incorporate many factors to determine the most appropriate tests to order. CSF volume from an LP should be conserved for use on tests of the most likely etiology, rather than ordering numerous tests from the start (a common practice in the era of electronic medical records and auto-populated order sets). Physicians must consider local epidemiology, duration of symptoms, current and recent medications, current immune status, country of origin, current living situation, social history, vaccination status and history of travel among other factors. Currently, excellent diagnostic techniques exist for cryptococcal meningitis along with many bacteria and viruses that cause meningitis. Yet significant room for improvement exists with other bacteria, viruses, fungi and TB. GeneXpert MTB/Rif Ultra seems to be a major improvement in the diagnosis of TBM, yet cases are still missed. Thus, additional improvements in TBM are urgently needed. Cutting-edge technologies such as NGS hold significant promise but require extensive bioinformatics and sample processing expertise – the role of NGS in meningitis diagnosis is not yet clear.

Future perspective

The field of diagnostic testing in infectious diseases is ever evolving. Impressive strides have been made over the last 5 years in NGS, and these technologies are starting to be used clinically. Newer molecular techniques that utilize the CRISPR-Cas9 system are in their infancy but may have potential with further investigation. It remains difficult to assess which new diagnostic techniques will become available in low income setting due to significant monetary and practical barriers for implementation. Yet, to truly improve diagnosis of meningitis worldwide, diagnostic test development must focus on these practical issues that affect test availability in many settings worldwide.

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Executive summary

Background

- Infectious meningitis may be due to bacteria, viruses, mycobacteria such as tuberculosis, or fungi (most notably *Cryptococcus neoformans*).
- Mortality rates are unacceptably high expect for in viral meningitis.
- Prompt diagnosis and treatment are keys to improving outcomes.

Epidemiology

- Incidence of meningitis due each infectious etiology varies by location, though *Sreptococcus pneumoniae* remains the most common cause of bacterial meningitis worldwide.
- *Cryptococcus* and *Mycobacterium tuberculosis* are much more common in areas with high rates of HIV and are the two most common causes of meningitis in sub-Saharan Africa.

General diagnostic tests

- Cerebrospinal fluid (CSF) White cell count and differential, total protein and glucose measurements from CSF are all helpful tools for diagnosing meningitis. Yet, despite 'classic' patterns, these nonspecific markers are not specific enough for definite diagnosis.
- Those with meningitis due to bacteria or mycobacteria, generally have more inflammatory CSF than those with viral or fungal meningitis.

Bacterial meningitis

- The mainstay of diagnosis for bacterial meningitis remains CSF culture.
- PCR testing, especially in the form of assays that test for multiple pathogens has the potential to reduce the time to diagnosis and thus allow for more rapid narrowing of antibiotics.
- MALDI-TOF MS is widely used for detection of bacteria in blood, and has been shown to have the potential for application to CSF samples and diagnosis of bacterial meningitis.

Tuberculosis meningitis

- While Acid-fast bacilli smear is fast and widely available, its sensitivity is inadequate (<15%).
- Culture is more sensitive, it still detects only 50–60% of TBM and results take multiple weeks.
- Xpert MTB/Rif (GeneXpert) is an automated PCR that can detect *M. tuberculosis* DNA while simultaneously testing for genes that correspond to rifampin resistance.
- Xpert MTB/Rif Ultra is more sensitive than Xpert or culture and is now recommended as the first test to be used to detect tuberculosis meningitis (TBM).

Cryptococcal meningitis

- Diagnosis of cryptococcal meningitis centers around detection of cryptococcal antigen (CrAg) via lateral flow assay, though culture is often helpful as well.
- Detection of CrAg in serum or plasma is recommended as a screening technique for treatment naive HIV patients with CD4 counts <100 cells/μl. If positive, fluconazole preemptive therapy is recommended to prevent cryptococcal meningitis.

Other fungi

- Diagnosis of other types of fungal meningitis remains difficult, and requires a high degree of clinical suspicion and awareness of geographic endemicity.
- Diagnosis of other forms of fungal meningitis has historically centered around fungal culture, which takes a significant amount of time and has low diagnostic yield.
- Fungus-specific antibody, antigen or non-specific 1,3-β-D-glucan testing has been utilized.
- Further development of rapid diagnostic techniques are needed, especially with the ever growing immunosuppressed patient population.

Aseptic meningitis

- Diagnostic techniques for aseptic meningitis, specifically PCR, may be underutilized and could lead to decreased antibiotic utilization.
- Others argue to only test for those viral etiologies that are treatable, such as Herpes Simplex Virus and defer testing for entities that do not have a targeted antiviral treatment, such as Enterovirus.

Conclusion & future perspective

- Early diagnosis and treatment remain imperative for good outcomes in meningitis.
- Diagnosis of cryptococcal meningitis is ideal, while bacterial meningitis diagnosis may be accurate if clinicians maintain a high degree of suspicion.
- GeneXpert MTB/Rif Ultra has the potential to significantly improve diagnosis of TBM but is not adequate to 'rule out' TBM.
- Next-generation sequencing techniques hold excellent potential for diagnosis of sub-acute and chronic meningitis in particular.

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References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Bahr NC, Boulware DR. Methods of rapid diagnosis for the etiology of meningitis in adults. *Biomark. Med.* 8(9), 1085–1103 (2014).
2. Davis LE. Acute bacterial meningitis. *Continuum (Minneap. Minn.)* 24(5, Neuroinfectious Disease), 1264–1283 (2018).
3. Bhimraj A. Acute community-acquired bacterial meningitis in adults: an evidence-based review. *Cleve. Clin. J. Med.* 79(6), 393–400 (2012).
4. Van Ettekoven CN, Van De Beek D, Brouwer MC. Update on community-acquired bacterial meningitis: guidance and challenges. *Clin. Microbiol. Infect.* 23(9), 601–606 (2017).
5. Charalambous LT, Premji A, Tybout C *et al.* Prevalence, healthcare resource utilization and overall burden of fungal meningitis in the United States. *J. Med. Microbiol.* 67(2), 215–227 (2018).
6. Mount HR, Boyle SD. Aseptic and bacterial meningitis: evaluation, treatment, and prevention. *Am. Fam. Phys.* 96(5), 314–322 (2017).
7. Adriani KS, Brouwer MC, Van De Beek D. Risk factors for community-acquired bacterial meningitis in adults. *Neth. J. Med.* 73(2), 53–60 (2015).
8. Amidu N, Antuamwine BB, Addai-Mensah O *et al.* Diagnosis of bacterial meningitis in Ghana: polymerase chain reaction versus latex agglutination methods. *PLoS ONE* 14(1), e0210812 (2019).
9. Houri H, Pormohammad A, Riahi SM *et al.* Acute bacterial meningitis in Iran: Systematic review and meta-analysis. *PLoS ONE* 12(2), e0169617 (2017).
10. Bahr NC, Nuwagira E, Evans EE *et al.* Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. *Lancet Infect. Dis.* 18(1), 68–75 (2018).

•• **The first study to describe the use of Xpert Ultra for diagnosis of tuberculous meningitis. When compared with Xpert MTB/RIF and culture Xpert Ultra was able to detect more cases of tuberculous meningitis.**

11. Abassi M, Boulware DR, Rhein J. Cryptococcal meningitis: diagnosis and management update. *Curr. Trop. Med. Rep.* 2(2), 90–99 (2015).
12. Rajasingham R, Smith RM, Park BJ *et al.* Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect. Dis.* 17(8), 873–881 (2017).
13. Antinori S, Corbellino M, Meroni L *et al. Aspergillus meningitis*: a rare clinical manifestation of central nervous system aspergillosis. Case report and review of 92 cases. *J. Infect.* 66(3), 218–238 (2013).
14. Stevens DA, Zhang Y, Finkelman MA, Pappagianis D, Clemons KV, Martinez M. Cerebrospinal fluid (1,3)-beta-d-glucan testing is useful in diagnosis of coccidioidal meningitis. *J. Clin. Microbiol.* 54(11), 2707–2710 (2016).
15. Wheat LJ, Batteiger BE, Sathapatayavongs B. Histoplasma capsulatum infections of the central nervous system. A clinical review. *Medicine (Baltimore)* 69(4), 244–260 (1990).
16. Sulaiman T, Salazar L, Hasbun R. Acute versus subacute community-acquired meningitis: analysis of 611 patients. *Medicine (Baltimore)* 96(36), e7984 (2017).
17. Mcgill F, Heyderman RS, Michael BD *et al.* The UK joint specialist societies guideline on the diagnosis and management of acute meningitis and meningococcal sepsis in immunocompetent adults. *J. Infect.* 72(4), 405–438 (2016).
18. Hasbun R, Rosenthal N, Balada-Llasat JM *et al.* Epidemiology of meningitis and encephalitis in the United States, 2011–2014. *Clin. Infect. Dis.* 65(3), 359–363 (2017).
19. Leber AL, Everhart K, Balada-Llasat JM *et al.* Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J. Clin. Microbiol.* 54(9), 2251–2261 (2016).

• **Describes a multicentered study utilizing the Biofire FilmArray Meningitis/Encephalitis Panel. This study found that the Biofire FilmArray was highly sensitive and specific with rapid results.**

20. Brouwer MC, Van De Beek D. Epidemiology of community-acquired bacterial meningitis. *Curr. Opin. Infect. Dis.* 31(1), 78–84 (2018).
21. Diallo AO, Soeters HM, Yameogo I *et al.* Bacterial meningitis epidemiology and return of *Neisseria meningitidis* serogroup A cases in Burkina Faso in the five years following MenAfriVac mass vaccination campaign. *PLoS ONE* 12(11), e0187466 (2017).
22. Jarrin I, Sellier P, Lopes A *et al.* Etiologies and management of aseptic meningitis in patients admitted to an internal medicine department. *Medicine (Baltimore)* 95(2), e2372 (2016).

23. Shukla B, Aguilera EA, Salazar L, Wootton SH, Kaewpoowat Q, Hasbun R. Aseptic meningitis in adults and children: diagnostic and management challenges. *J. Clin. Virol.* 94, 110–114 (2017).
24. Thao LTP, Heemskerk AD, Geskus RB *et al.* Prognostic models for 9-month mortality in tuberculous meningitis. *Clin. Infect. Dis.* 66(4), 523–532 (2018).
25. World Health Organization. Global Tuberculosis Report 2017 (2017). www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf
26. Rajasingham R, Rhein J, Klammer K *et al.* Epidemiology of meningitis in an HIV-infected Ugandan cohort. *Am. J. Trop. Med. Hyg.* 92(2), 274–279 (2015).
27. Zunt JR, Baldwin KJ. Chronic and subacute meningitis. *Continuum (Minneap. Minn.)* 18(6 Infectious Disease), 1290–1318 (2012).
28. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. Histoplasmosis. *Infect. Dis. Clin. North. Am.* 30(1), 207–227 (2016).
29. Lundbo LF, Benfield T. Risk factors for community-acquired bacterial meningitis. *Infect. Dis. (Lond.)* 49(6), 433–444 (2017).
30. Mezochow A, Thakur K, Vinnard C. Tuberculous meningitis in children and adults: new insights for an ancient foe. *Curr. Neurol. Neurosci. Rep.* 17(11), 85 (2017).
31. Deisenhammer F, Bartos A, Egg R *et al.* Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force. *Eur. J. Neurol.* 13(9), 913–922 (2006).
32. Piquet AL, Lyons JL. Infectious meningitis and encephalitis. *Semin. Neurol.* 36(4), 367–372 (2016).
33. Pehlivanoglu F, Yasar KK, Sengoz G. Tuberculous meningitis in adults: a review of 160 cases. *ScientificWorldJournal* 2012 , 169028 (2012).
34. Abreu C, Magro F, Vilas-Boas F, Lopes S, Macedo G, Sarmento A. Listeria infection in patients on anti-TNF treatment: report of two cases and review of the literature. *J. Crohns Colitis* 7(2), 175–182 (2013).
35. Nobre CA, Callado MR, Lima JR, Gomes KW, Martiniano GV, Vieira WP. Tuberculosis infection in rheumatic patients with infliximab therapy: experience with 157 patients. *Rheumatol. Int.* 32(9), 2769–2775 (2012).
36. Keane J, Gershon S, Wise RP *et al.* Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N. Engl. J. Med.* 345(15), 1098–1104 (2001).
37. Downey C. Serious infection during etanercept, infliximab and adalimumab therapy for rheumatoid arthritis: a literature review. *Int. J. Rheum. Dis.* 19(6), 536–550 (2016).
38. Rana F, Shaikh MM, Bowles J. Listeria meningitis and resultant symptomatic hydrocephalus complicating infliximab treatment for ulcerative colitis. *JRSM Open* 5(3), 2054270414522223 (2014).
39. Van Veen KE, Brouwer MC, Van Der Ende A, Van De Beek D. Bacterial meningitis in solid organ transplant recipients: a population-based prospective study. *Transpl. Infect. Dis.* 18(5), 674–680 (2016).
40. Gassiep I, Aye C, Armstrong M, Emeto TI, Heather CS, Norton RE. Correlation between serum cryptococcal antigen titre and meningitis in immunocompetent patients. *J. Med. Microbiol.* 67(10), 1515–1518 (2018).
41. Singh N, Sifri CD, Silveira FP *et al.* Cryptococcosis in patients with cirrhosis of the liver and posttransplant outcomes. *Transplantation* 99(10), 2132–2141 (2015).
42. Van Veen KE, Brouwer MC, Van Der Ende A, Van De Beek D. Bacterial meningitis in hematopoietic stem cell transplant recipients: a population-based prospective study. *Bone Marrow Transplant.* 51(11), 1490–1495 (2016).
43. Desai SD, Seth S, Shah A, Vaishnav B. *Aspergillus meningoencephalitis* in a patient with human immunodeficiency virus infection: normal cerebrospinal fluid does not mean absence of meningitis. *Indian J. Sex Transm. Dis. AIDS* 37(2), 185–189 (2016).
44. Klont RR, Mennink-Kersten MA, Verweij PE. Utility of *Aspergillus* antigen detection in specimens other than serum specimens. *Clin. Infect. Dis.* 39(10), 1467–1474 (2004).
45. Badiee P, Alborzi A. Assessment of a real-time PCR method to detect human non-cryptococcal fungal meningitis. *Arch. Iran Med.* 14(6), 381–384 (2011).
46. Richie MB, Josephson SA. A practical approach to meningitis and encephalitis. *Semin. Neurol.* 35(6), 611–620 (2015).
47. Kaur H, Sharma K, Modi M *et al.* Prospective analysis of 55 cases of tuberculosis meningitis (TBM) in North India. *J. Clin. Diagn. Res.* 9(1), DC15-19 (2015).
48. Madigan T, Fatemi Y, Theel ES, Moodley A, Boyce TG. Central nervous system blastomycosis in children: a case report and review of the literature. *Pediatr. Infect. Dis. J.* 36(7), 679–684 (2017).
49. Bush JW, Wuerz T, Embil JM, Del Bigio MR, Mcdonald PJ, Krawitz S. Outcomes of persons with blastomycosis involving the central nervous system. *Diagn. Microbiol. Infect. Dis.* 76(2), 175–181 (2013).
50. Glimaker M, Johansson B, Grindborg O, Bottai M, Lindquist L, Sjolin J. Adult bacterial meningitis: earlier treatment and improved outcome following guideline revision promoting prompt lumbar puncture. *Clin. Infect. Dis.* 60(8), 1162–1169 (2015).
51. Shokrollahi MR, Shabanzadeh K, Noorbakhsh S, Tabatabaei A, Movahedi Z, Shamshiri AR. Diagnostic value of CRP, procalcitonin, and ferritin levels in cerebrospinal fluid of children with meningitis. *Cent. Nerv. Syst. Agents Med. Chem.* 18(1), 58–62 (2018).

52. Tunkel AR, Hartman BJ, Kaplan SL *et al.* Practice guidelines for the management of bacterial meningitis. *Clin. Infect. Dis.* 39(9), 1267–1284 (2004).
53. Buch K, Bodilsen J, Knudsen A *et al.* Cerebrospinal fluid lactate as a marker to differentiate between community-acquired acute bacterial meningitis and aseptic meningitis/encephalitis in adults: a Danish prospective observational cohort study. *Infect. Dis. (Lond.)* 50(7), 514–521 (2018).
54. Kumar A, Debata PK, Ranjan A, Gaind R. The role and reliability of rapid bedside diagnostic test in early diagnosis and treatment of bacterial meningitis. *Indian J. Pediatr.* 82(4), 311–314 (2015).
55. Mohammadi SF, Patil AB, Nadagir SD, Nandihal N, Lakshminarayana SA. Diagnostic value of latex agglutination test in diagnosis of acute bacterial meningitis. *Ann. Indian Acad. Neurol.* 16(4), 645–649 (2013).
56. Al Zamil F, Al Mazrou A, Qureshi IM, Al Ayed I, Kambal AM. Pattern of pathogens and utility of latex agglutination tests in bacterial meningitis. *Ann. Saudi Med.* 22(5–6), 388–391 (2002).
57. Whittle HC, Tugwell P, Egler LJ, Greenwood BM. Rapid bacteriological diagnosis of pyogenic meningitis by latex agglutination. *Lancet* 2(7881), 619–621 (1974).
58. Habte-Gabr E, Muhe L, Olcen P. Rapid etiological diagnosis of pyogenic meningitis by coagglutination, latex agglutination and immunoosmophoresis of cerebrospinal fluid, serum and urine. *Trop. Geogr. Med.* 39(2), 137–143 (1987).
59. Paliwal VK, Tejan N. Clinical utility of latex agglutination test for the rapid diagnosis of acute bacterial meningitis. *Neurol. India* 66(4), 1050–1051 (2018).
60. Jorgensen JH, Lee JC. Rapid diagnosis of gram-negative bacterial meningitis by the Limulus endotoxin assay. *J. Clin. Microbiol.* 7(1), 12–17 (1978).
61. Siegel SE, Nachum R. Indications for the use of the Limulus lysate assay in bacterial meningitis. *Prog. Clin. Biol. Res.* 29, 245–253 (1979).
62. Bhagchandani SP, Kubade S, Nikhare PP *et al.* Nested PCR assay for eight pathogens: a rapid tool for diagnosis of bacterial meningitis. *Mol. Diagn. Ther.* 20(1), 45–54 (2016).
63. Baspinar EO, Dayan S, Bekcibasi M *et al.* Comparison of culture and PCR methods in the diagnosis of bacterial meningitis. *Braz. J. Microbiol.* 48(2), 232–236 (2017).
64. Uzuka R, Kawashima H, Hasegawa D *et al.* Rapid diagnosis of bacterial meningitis by using multiplex PCR and real time PCR. *Pediatr. Int.* 46(5), 551–554 (2004).
65. Welinder-Olsson C, Dotevall L, Hogevik H *et al.* Comparison of broad-range bacterial PCR and culture of cerebrospinal fluid for diagnosis of community-acquired bacterial meningitis. *Clin. Microbiol. Infect.* 13(9), 879–886 (2007).
66. Ao D, Wei L, Hui-Hui G, Ran T, Shi-Qiang S, Yue-Li R. Rapid diagnosis and discrimination of bacterial meningitis in children using gram probe real-time polymerase chain reaction. *Clin. Pediatr. (Phila.)* 53(9), 839–844 (2014).
67. Wang Y, Guo G, Wang H *et al.* Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis. *BMC Pediatr.* 14, 224 (2014).
68. Albuquerque RC, Moreno ACR, Dos Santos SR, Ragazzi SLB, Martinez MB. Multiplex-PCR for diagnosis of bacterial meningitis. *Braz. J. Microbiol.* doi:10.1007/s42770-019-00055-9 (2019) (Epub ahead of print).
69. Wagner K, Springer B, Pires VP, Keller PM. Pathogen identification by multiplex lightmix real-time PCR assay in patients with meningitis and culture-negative cerebrospinal fluid specimens. *J. Clin. Microbiol.* 56(2), (2018).
70. Khumalo J, Nicol M, Hardie D, Muloiwa R, Mteshana P, Bamford C. Diagnostic accuracy of two multiplex real-time polymerase chain reaction assays for the diagnosis of meningitis in children in a resource-limited setting. *PLoS ONE* 12(3), e0173948 (2017).
71. Wootton SH, Aguilera E, Salazar L, Hemmert AC, Hasbun R. Enhancing pathogen identification in patients with meningitis and a negative Gram stain using the BioFire FilmArray((R)) meningitis/encephalitis panel. *Ann. Clin. Microbiol. Antimicrob.* 15, 26 (2016).
72. Kim DW, Kilgore PE, Kim EJ, Kim SA, Anh DD, Seki M. Loop-mediated isothermal amplification assay for detection of Haemophilus influenzae type b in cerebrospinal fluid. *J. Clin. Microbiol.* 49(10), 3621–3626 (2011).
73. Higgins O, Clancy E, Cormican M, Boo TW, Cunney R, Smith TJ. Evaluation of an internally controlled multiplex Tth endonuclease cleavage loop-mediated isothermal amplification (TEC-LAMP) assay for the detection of bacterial meningitis pathogens. *Int. J. Mol. Sci.* 19(2), pii: E524. (2018).
74. Seki M, Kilgore PE, Kim EJ, Ohnishi M, Hayakawa S, Kim DW. Loop-mediated isothermal amplification methods for diagnosis of bacterial meningitis. *Front. Pediatr.* 6, 57 (2018).
75. Dou M, Sanjay ST, Dominguez DC, Liu P, Xu F, Li X. Multiplexed instrument-free meningitis diagnosis on a polymer/paper hybrid microfluidic biochip. *Biosens. Bioelectron.* 87, 865–873 (2017).
76. Batista AC, Pacheco LGC. Detecting pathogens with Zinc-Finger, TALE and CRISPR- based programmable nucleic acid binding proteins. *J. Microbiol. Methods* 152, 98–104 (2018).
77. Gronowski AM. Who or what is SHERLOCK? *EJIFCC* 29(3), 201–204 (2018).
78. Caliendo AM, Hodinka RL. A CRISPR way to diagnose infectious diseases. *N. Engl. J. Med* .377(17), 1685–1687 (2017).

79. Barrangou R, Horvath P. A decade of discovery: CRISPR functions and applications. *Nat. Microbiol.* 2, 17092 (2017).

80. Gootenberg JS, Abudayyeh OO, Lee JW *et al.* Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* 356(6336), 438–442 (2017).

- **CRISPR-based diagnostics are in their infancy and have potential utility as rapid, point-of care technique to identify pathogens. This study describes the use of CRISPR-based diagnostics to distinguish bacterial pathogens.**

81. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science* 360(6387), 439–444 (2018).

82. 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.* 78(5), 409–421 (2019).

83. Guo LY, Li YJ, Liu LL *et al.* Detection of pediatric bacterial meningitis pathogens from cerebrospinal fluid by next-generation sequencing technology. *J. Infect.* 78(4), 323–337 (2019).

84. Wilson MR, O'donovan BD, Gelfand JM *et al.* Chronic meningitis investigated via metagenomic next-generation sequencing. *JAMA Neurol* 75(8), 947–955 (2018).

- **Next-generation sequencing (NGS) is an evolving field, this paper describes its use in the diagnosis of subacute and chronic meningitis. In this report four cases of fungal meningitis were diagnosed using NGS.**

85. Wilson MR, Sample HA, Zorn KC *et al.* Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. *N. Engl. J. Med.* 380(24), 2327–2340 (2019).

- **NGS has the potential to improve diagnosis of meningitis and encephalitis. In this study, NGS was able to identify 13 infections that were not identified by other means of testing.**

86. Nyvang Hartmeyer G, Kvistholm Jensen A, Bocher S *et al.* Mass spectrometry: pneumococcal meningitis verified and *Brucella* species identified in less than half an hour. *Scand. J. Infect. Dis.* 42(9), 716–718 (2010).

87. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol. Rev.* 36(2), 380–407 (2012).

88. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. *Clin Chem* 61(1), 100–111 (2015).

89. 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* 435, 59–61 (2014).

- **MALDI-TOF testing is routinely used for identification of organism from blood cultures. This case report describes a patient who was diagnosed with bacterial meningitis by using MALDI-TOF on CSF.**

90. Dendane T, Madani N, Zekraoui A *et al.* A simple diagnostic aid for tuberculous meningitis in adults in Morocco by use of clinical and laboratory features. *Int. J. Infect. Dis.* 17(6), e461–465 (2013).

91. Zhang P, Zhang W, Lang Y *et al.* Mass spectrometry-based metabolomics for tuberculosis meningitis. *Clin. Chim. Acta* 483, 57–63 (2018).

92. Zhou XX, Liu YL, Zhai K, Shi HZ, Tong ZH. Body Fluid interferon-gamma release assay for diagnosis of extrapulmonary tuberculosis in adults: a systematic review and meta-analysis. *Sci. Rep.* 5, 15284 (2015).

93. Gunes A, Uluca U, Aktar F *et al.* Clinical, radiological and laboratory findings in 185 children with tuberculous meningitis at a single centre and relationship with the stage of the disease. *Ital. J. Pediatr.* 41, 75 (2015).

94. Mai NT, Thwaites GE. Recent advances in the diagnosis and management of tuberculous meningitis. *Curr. Opin. Infect. Dis.* 30(1), 123–128 (2017).

95. Chen P, Shi M, Feng GD *et al.* A highly efficient Ziehl-Neelsen stain: identifying de novo intracellular *Mycobacterium tuberculosis* and improving detection of extracellular *M. tuberculosis* in cerebrospinal fluid. *J. Clin. Microbiol.* 50(4), 1166–1170 (2012).

96. Galimi R. Extrapulmonary tuberculosis: tuberculous meningitis new developments. *Eur. Rev. Med. Pharmacol. Sci.* 15(4), 365–386 (2011).

97. Torok ME. Tuberculous meningitis: advances in diagnosis and treatment. *Br. Med. Bull.* 113(1), 117–131 (2015).

98. Ho J, Marais BJ, Gilbert GL, Ralph AP. Diagnosing tuberculous meningitis - have we made any progress? *Trop. Med. Int. Health* 18(6), 783–793 (2013).

99. Thwaites GE. Advances in the diagnosis and treatment of tuberculous meningitis. *Curr. Opin. Neurol.* 26(3), 295–300 (2013).

100. Shekhawat SD, Purohit HJ, Taori GM, Daginawala HF, Kashyap RS. Evaluation of host Hsp(s) as potential biomarkers for the diagnosis of tuberculous meningitis. *Clin. Neurol. Neurosurg.* 140, 47–51 (2016).

101. Coovadia YM, Dawood A, Ellis ME, Coovadia HM, Daniel TM. Evaluation of adenosine deaminase activity and antibody to *Mycobacterium tuberculosis* antigen 5 in cerebrospinal fluid and the radioactive bromide partition test for the early diagnosis of tuberculosis meningitis. *Arch. Dis. Child* 61(5), 428–435 (1986).

102. Xu HB, Jiang RH, Li L, Sha W, Xiao HP. Diagnostic value of adenosine deaminase in cerebrospinal fluid for tuberculous meningitis: a meta-analysis. *Int. J. Tuberc. Lung Dis.* 14(11), 1382–1387 (2010).

103. Bahr NC, Meintjes G, Boulware DR. Inadequate diagnostics: the case to move beyond the bacilli for detection of meningitis due to *Mycobacterium tuberculosis*. *J. Med. Microbiol.* 68(5), 755–760 (2019).

104. Bahr NC, Halupnick R, Linder G *et al.* Delta-like 1 protein, vitamin D binding protein and fetuin for detection of *Mycobacterium tuberculosis* meningitis. *Biomark. Med.* 12(7), 707–716 (2018).

105. Patel VB, Theron G, Lenders L *et al.* Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study. *PLoS Med.* 10(10), e1001536 (2013).

106. Nhu NT, Heemskerk D, Thu Do DA *et al.* Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. *J. Clin. Microbiol.* 52(1), 226–233 (2014).

107. Bahr NC, Tugume L, Rajasingham R *et al.* Improved diagnostic sensitivity for tuberculous meningitis with Xpert((R)) MTB/RIF of centrifuged CSF. *Int. J. Tuberc. Lung Dis.* 19(10), 1209–1215 (2015).

108. Trebucq A, Enarson DA, Chiang CY *et al.* Xpert(R) MTB/RIF for national tuberculosis programmes in low-income countries: when, where and how? *Int. J. Tuberc. Lung Dis.* 15(12), 1567–1572 (2011).

109. Bahr NC, Marais S, Caws M *et al.* GeneXpert MTB/Rif to diagnose tuberculous meningitis: perhaps the first test but not the last. *Clin. Infect. Dis.* 62(9), 1133–1135 (2016).

110. Wu X, Tan G, Gao R *et al.* Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis. *Int. J. Infect. Dis.* 81, 91–96 (2019).

111. Chakravorty S, Simmons AM, Rowneki M *et al.* The New Xpert MTB/RIf ultra: improving detection of *Mycobacterium tuberculosis* and resistance to Rifampin in an assay suitable for point-of-care testing. *MBio* 8(4), (2017).

112. Patel VB, Connolly C, Singh R *et al.* Comparison of amplicor and GeneXpert MTB/RIF tests for diagnosis of tuberculous meningitis. *J. Clin. Microbiol.* 52(10), 3777–3780 (2014).

113. Jonsson B, Ridell M. The Cobas Amplicor MTB test for detection of *Mycobacterium tuberculosis* complex from respiratory and non-respiratory clinical specimens. *Scand. J. Infect. Dis.* 35(6–7), 372–377 (2003).

114. Bonington A, Strang JI, Klapper PE *et al.* TB PCR in the early diagnosis of tuberculous meningitis: evaluation of the Roche semi-automated COBAS Amplicor MTB test with reference to the manual Amplicor MTB PCR test. *Tuber Lung Dis.* 80(4-5), 191–196 (2000).

115. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM, Jr. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infect. Dis.* 3(10), 633–643 (2003).

116. Causse M, Ruiz P, Gutierrez-Aroca JB, Casal M. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. *J. Clin. Microbiol.* 49(8), 3065–3067 (2011).

117. Alcala L, Ruiz-Serrano MJ, Hernangomez S *et al.* Evaluation of the upgraded amplified *Mycobacterium tuberculosis* direct test (gen-probe) for direct detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory specimens. *Diagn. Microbiol. Infect. Dis.* 41(1-2), 51–56 (2001).

118. Ehlers S, Ignatius R, Regnath T, Hahn H. Diagnosis of extrapulmonary tuberculosis by Gen-Probe amplified *Mycobacterium tuberculosis* direct test. *J. Clin. Microbiol.* 34(9), 2275–2279 (1996).

119. Modi M, Sharma K, Sharma M *et al.* Multitargeted loop-mediated isothermal amplification for rapid diagnosis of tuberculous meningitis. *Int. J. Tuberc. Lung Dis* 20(5), 625–630 (2016).

120. Patel VB, Bhigjee AI, Paruk HF *et al.* Utility of a novel lipoarabinomannan assay for the diagnosis of tuberculous meningitis in a resource-poor high-HIV prevalence setting. *Cerebrospinal Fluid Res.* 6, 13 (2009).

121. Cox JA, Lukande RL, Kalungi S *et al.* Accuracy of lipoarabinomannan and Xpert MTB/RIF testing in cerebrospinal fluid to diagnose tuberculous meningitis in an autopsy cohort of HIV-infected adults. *J. Clin. Microbiol.* 53(8), 2667–2673 (2015).

122. Bahr NC, Tugume L, Boulware DR. A word of caution in considering the use of the lipoarabinomannan lateral flow assay on cerebrospinal fluid for detection of tuberculous meningitis. *J. Clin. Microbiol.* 54(1), 241–242 (2016).

123. Shao Y, Xia P, Zhu T *et al.* Sensitivity and specificity of immunocytochemical staining of mycobacterial antigens in the cytoplasm of cerebrospinal fluid macrophages for diagnosing tuberculous meningitis. *J. Clin. Microbiol.* 49(9), 3388–3391 (2011).

124. Myint T, Chow FC, Bloch KC *et al.* Detection of (1,3)-beta-d-glucan in cerebrospinal fluid in histoplasma meningitis. *J. Clin. Microbiol.* 56(10), (2018).

125. Rhein J, Bahr NC, Morawski BM *et al.* Detection of high cerebrospinal fluid levels of (1–>3)-beta-d-glucan in cryptococcal meningitis. *Open Forum Infect. Dis.* 1(3), ofu105 (2014).

126. Rhein J, Boulware DR, Bahr NC. 1,3-beta-D-glucan in cryptococcal meningitis. *Lancet Infect. Dis.* 15(10), 1136–1137 (2015).

127. Ramachandran PS, Cresswell FV, Meya DB *et al.* Detection of cryptococcus DNA by metagenomic next-generation sequencing in symptomatic cryptococcal antigenemia. *Clin. Infect. Dis.* 68(11), 1978–1979 (2019).

128. Bloch KC, Myint T, Raymond-Guillen L *et al.* improvement in diagnosis of histoplasma meningitis by combined testing for histoplasma antigen and immunoglobulin G and immunoglobulin M anti-histoplasma antibody in cerebrospinal fluid. *Clin. Infect. Dis.* 66(1), 89–94 (2018).

129. Wheat LJ, Kohler RB, Tewari RP, Garten M, French ML. Significance of Histoplasma antigen in the cerebrospinal fluid of patients with meningitis. *Arch. Intern. Med.* 149(2), 302–304 (1989).

130. Nyalakonda H, Albuerne M, Suazo Hernandez LP, Sarria JC. Central nervous system histoplasmosis in acquired immunodeficiency syndrome. *Am. J. Med. Sci.* 351(2), 177–186 (2016).

131. Wheat LJ. Current diagnosis of histoplasmosis. *Trends Microbiol.* 11(10), 488–494 (2003).

132. Wheat LJ, Musial CE, Jenny-Avital E. Diagnosis and management of central nervous system histoplasmosis. *Clin. Infect. Dis.* 40(6), 844–852 (2005).

133. Schestatsky P, Chedid MF, Amaral OB, Unis G, Oliveira FM, Severo LC. Isolated central nervous system histoplasmosis in immunocompetent hosts: a series of 11 cases. *Scand J. Infect. Dis.* 38(1), 43–48 (2006).

134. Wheat J, Myint T, Guo Y *et al.* Central nervous system histoplasmosis: multicenter retrospective study on clinical features, diagnostic approach and outcome of treatment. *Medicine (Baltimore)* 97(13), e0245 (2018).

135. Richer SM, Smedema ML, Durkin MM *et al.* Improved diagnosis of acute pulmonary histoplasmosis by combining antigen and antibody detection. *Clin. Infect. Dis.* 62(7), 896–902 (2016).

136. Hage CA, Ribes JA, Wengenack NL *et al.* A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin. Infect. Dis.* 53(5), 448–454 (2011).

137. Bahr NC, Panackal AA, Durkin MM *et al.* Cryptococcal meningitis is a cause for cross-reactivity in cerebrospinal fluid assays for anti-Histoplasma, anti-Coccidioides and anti-Blastomyces antibodies. *Mycoses* 62(3), 268–273 (2019).

138. Kassis C, Zaidi S, Kuberski T *et al.* Role of coccidioides antigen testing in the cerebrospinal fluid for the diagnosis of coccidioidal meningitis. *Clin. Infect. Dis.* 61(10), 1521–1526 (2015).

139. Johnson RH, Einstein HE. Coccidioidal meningitis. *Clin. Infect. Dis.* 42(1), 103–107 (2006).

140. Williams PL. Coccidioidal meningitis. *Ann. NY Acad. Sci.* 1111, 377–384 (2007).

141. Bamberger DM, Pepito BS, Proia LA *et al.* Cerebrospinal fluid Coccidioides antigen testing in the diagnosis and management of central nervous system coccidioidomycosis. *Mycoses* 58(10), 598–602 (2015).

142. Blair JE. Coccidioidal meningitis: update on epidemiology, clinical features, diagnosis, and management. *Curr. Infect. Dis. Rep* 11(4), 289–295 (2009).

143. Johnson R, Ho J, Fowler P, Heidari A. Coccidioidal meningitis: a review on diagnosis, treatment, and management of complications. *Curr. Neurol. Neurosci. Rep.* 18(4), 19 (2018).

144. Mchardy IH, Dinh BN, Waldman S *et al.* Coccidioidomycosis complement fixation titer trends in the age of antifungals. *J. Clin. Microbiol.* 56(12), doi:10.1128/JCM.01318-18 (2018).

145. Stockamp NW, Thompson GR, 3rd. Coccidioidomycosis. *Infect. Dis. Clin. North Am.* 30(1), 229–246 (2016).

146. Vidal JE, Boulware DR. Lateral flow assay for cryptococcal antigen: an important advance to improve the continuum of HIV care and reduce cryptococcal meningitis-related mortality. *Rev. Inst. Med. Trop. Sao Paulo* 57(Suppl. 19), 38–45 (2015).

147. Boulware DR, Rolfes MA, Rajasingham R *et al.* Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. *Emerg. Infect. Dis.* 20(1), 45–53 (2014).

148. Williamson PR, Jarvis JN, Panackal AA *et al.* Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy. *Nat. Rev. Neurol.* 13(1), 13–24 (2017).

149. Rhein J, Bahr NC, Hemmert AC *et al.* Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn. Microbiol. Infect. Dis.* 84(3), 268–273 (2016).

150. Liesman RM, Strasburg AP, Heitman AK, Theel ES, Patel R, Binnicker MJ. Evaluation of a commercial multiplex molecular panel for diagnosis of infectious meningitis and encephalitis. *J. Clin. Microbiol.* 56(4), doi:10.1128/JCM.01927-17 (2018).

151. Ramachandran A, Manabe Y, Rajasingham R, Shah M. Cost-effectiveness of CRAG-LFA screening for cryptococcal meningitis among people living with HIV in Uganda. *BMC Infect. Dis.* 17(1), 225 (2017).

152. Kabanda T, Siedner MJ, Klausner JD, Muzoora C, Boulware DR. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal antigen lateral flow assay on cerebrospinal fluid. *Clin. Infect. Dis.* 58(1), 113–116 (2014).

153. Williams DA, Kiiza T, Kwizera R *et al.* Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: a diagnostic accuracy study. *Clin. Infect. Dis.* 61(3), 464–467 (2015).

154. Vidal JE, Toniolo C, Paulino A *et al.* Performance of cryptococcal antigen lateral flow assay in serum, cerebrospinal fluid, whole blood, and urine in HIV-infected patients with culture-proven cryptococcal meningitis admitted at a Brazilian referral center. *Rev. Inst. Med. Trop. Sao Paulo* 60, e1 (2018).

155. Jarvis JN, Percival A, Bauman S *et al.* Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clin. Infect. Dis.* 53(10), 1019–1023 (2011).

156. Lourens A, Jarvis JN, Meintjes G, Samuel CM. Rapid diagnosis of cryptococcal meningitis by use of lateral flow assay on cerebrospinal fluid samples: influence of the high-dose "hook" effect. *J. Clin. Microbiol.* 52(12), 4172–4175 (2014).

157. Dubbels M, Granger D, Theel ES. Low cryptococcus antigen titers as determined by lateral flow assay should be interpreted cautiously in patients without prior diagnosis of cryptococcal infection. *J. Clin. Microbiol.* 55(8), 2472–2479 (2017).

158. Ssebambulidde K, Bangdiwala AS, Kwizera R *et al.* Symptomatic cryptococcal antigenemia presenting as early cryptococcal meningitis with negative CSF analysis. *Clin. Infect. Dis.* doi:10.1093/cid/ciy817 (2018) (Epub ahead of print).

159. Meya DB, Manabe YC, Castelnuovo B *et al.* Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a $CD4^+$ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. *Clin. Infect. Dis.* 51(4), 448–455 (2010).

160. Walkty A, Keynan Y, Karlowsky J, Dhaliwal P, Embil J. Central nervous system blastomycosis diagnosed using the MVista(R) Blastomyces quantitative antigen enzyme immunoassay test on cerebrospinal fluid: a case report and review of the literature. *Diagn. Microbiol. Infect. Dis.* 90(2), 102–104 (2018).

161. Bariola JR, Hage CA, Durkin M *et al.* Detection of Blastomyces dermatitidis antigen in patients with newly diagnosed blastomycosis. *Diagn. Microbiol. Infect. Dis.* 69(2), 187–191 (2011).

162. Brown DA, Whealy MA, Van Gompel JJ, Williams LN, Klaas JP. Diagnostic dilemma in primary Blastomyces dermatitidis meningitis: role of neurosurgical biopsy. *Case Rep. Neurol.* 7(1), 63–70 (2015).

163. Bariola JR, Perry P, Pappas PG *et al.* Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin. Infect. Dis.* 50(6), 797–804 (2010).

164. Saccente M, Woods GL. Clinical and laboratory update on blastomycosis. *Clin. Microbiol. Rev.* 23(2), 367–381 (2010).

165. Frost HM, Novicki TJ. Blastomyces antigen detection for diagnosis and management of blastomycosis. *J. Clin. Microbiol.* 53(11), 3660–3662 (2015).

166. Friedman JA, Wijdicks EF, Fulgham JR, Wright AJ. Meningoencephalitis due to *Blastomyces dermatitidis*: case report and literature review. *Mayo Clin .Proc.* 75(4), 403–408 (2000).

167. Verweij PE, Brinkman K, Kremer HP, Kullberg BJ, Meis JF. *Aspergillus meningitis*: diagnosis by non-culture-based microbiological methods and management. *J. Clin. Microbiol.* 37(4), 1186–1189 (1999).

168. Kourkoumpetis TK, Desalermos A, Muhammed M, Mylonakis E. Central nervous system aspergillosis: a series of 14 cases from a general hospital and review of 123 cases from the literature. *Medicine (Baltimore)* 91(6), 328–336 (2012).

169. Soeffker G, Wichmann D, Loderstaedt U, Sobottka I, Deuse T, Kluge S. *Aspergillus galactomannan* antigen for diagnosis and treatment monitoring in cerebral aspergillosis. *Prog. Transplant.* 23(1), 71–74 (2013).

170. Sakushima K, Hayashino Y, Kawaguchi T, Jackson JL, Fukuhara S. Diagnostic accuracy of cerebrospinal fluid lactate for differentiating bacterial meningitis from aseptic meningitis: a meta-analysis. *J. Infect.* 62(4), 255–262 (2011).