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RESEARCH ARTICLE

LABORATORY DIAGNOSIS OF NEUROINFECTIOUS DISEASES- BASIC AND ADVANCE TESTS: MINI REVIEW

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Abstract

Cerebrospinal fluid (CSF) analysis is the mainstay basic test for the diagnosis of central nervous system (CNS) infections. However advance tests like CSF molecular testing in combination with other basic tests, both in CSF and blood are important in the approach to diagnosis of CNS infections. Nowadays multiplex molecular tests have been developed which have less turnaround time enabling rapid reporting to the clinicians. This review has been done to discuss the basic and advance tests available for diagnosing neuroinfectious diseases. The source of data is from medline database search and google search engine and reference textbooks. New molecular multiplex panels like BIOFIRE assay have been developed to simultaneously detect a large array of neuropathogens in CSF. Detailed list of neuropathogens have been mentioned in this review with advantages/disadvantages of all basic and advance tests available to diagnose them in a tabulated format . A flowchart on the stepwise testing approach to diagnose CNS infections has been given. Laboratory diagnosis of neuroinfectious diseases- basic and advance tests: Mini review.

Introduction:-

Diagnosis of neurological infections still remain a major challenge. Moreover, new emerging infections like West Nile, Hanta, Marburg, severe acute respiratory syndrome, the Ebola virus, Zika virus etc. , spread fast globally in record time due to easy travel nowadays. [1,2] Many infections remain undiagnosed and their burden in the society is under reported. Even today in this era of polymerase chain reaction (PCR) , etiological diagnosis is not established in approximately 30% of patients with a suspected CNS infection despite prolonged hospital stay and several investigations. [1] Non specific clinical symptoms like fever, headache, vomiting, photophobia, stiff neck, and focal neurological presentations may indicate towards a CNS infection. [3] PCR - based molecular methods are now widely used for routine microbial detection because in comparison to conventional techniques, they have rapid turnaround time with higher sensitivity and specificity thus making them the new gold standard for diagnosis of CNS infection especially for pathogens that are difficult to detect by the conventional culture and serology methods. [3]

Table 1:- Broad Classification of neurological infections [3,4]

Based on Anatomic location	Type of infection	Duration of infection
Localized – Meninges	Meningitis	acute, sub-acute, chronic, or

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Brain Spinal cord	Encephalitis Brain abscess Myelitis	recurrent
Multiple site – Meninges and brain Brain and spinal cord	Meningoencephalitis Encephalomyelitis	acute, sub-acute, chronic, or recurrent

Important factors to be considered before ordering a test for suspected neurological infection –

1. Geographical location and Epidemiology of the suspected infection
2. Clinical presentation
3. Host immune status
4. Animal exposure history
5. Vector exposure history
6. Travel history
7. Seasonality
8. Exposure to sick contacts
9. Any occupational hazard
10. Antimicrobial use history

Basic and advance Laboratory tests for diagnosis of neuroinfectious diseases:

Cerebrospinal fluid (CSF) analysis is the main key to specific etiological diagnosis of neurological infections.. Nonspecific testing like CSF analysis (refer to table 3) when correlated with clinical condition can guide to identify the more likely etiologies. Selection of specific laboratory tests and clinical samples to be sent for targeted microbial testing requires close communication between clinician and lab personnel. The clinician should inform the lab about most likely infection suspected by them based on clinical presentation so that the lab can take special measures to isolate and identify the pathogen. Also the lab should alert the clinician as soon as a presumptive identification is made so as to initiate appropriate treatment.

Factors that influence the performance of a test-

1. Standard precautions – should be implemented to prevent contamination
2. Appropriate site and sample type- for appropriate clinical correlation with the test result.
3. Timeline of sample collection – this is important especially for acute infections where for culture/ direct antigen assays/PCR organism might only be present in the CNS/blood for the first few hours or days of illness.
4. Proper sample collection technique – is important to prevent contamination and for result interpretation.
5. Sample sent in proper container- sterility should be maintained especially for culture.
6. Transport and storage- very important for correct results.

Advance Laboratory tests are based on molecular methods (table 5) like PCR. They are especially useful for viral/ fungal/other rare pathogens that are difficult to identify by basic lab tests. Another recent advancement is Metagenomic next-generation sequencing (mNGS) which is now a clinically validated test for neuroinfectious diseases and clinicians can make timely diagnosis especially in cases where detection of pathogens were missed by clinicians or by standard direct testing.

Table 2:- List of common pathogens associated with neurological infections based on anatomic location [3,4]

Anatomic location	Pathogen name
Brain Parenchyma	Bacteria- • Listeria monocytogenes • Nocardia • Ehrlichia • Bartonella Fungi- • Cryptococcus neoformans • Blastocystis hominis • Coccidioidis immitis

	• Aspergillus species • Zygomycetes Parasitic- • Toxoplasma gondii • Acanthamoeba • Balamuthia • Cerebral malaria • Neurocysticercois
Microglia	HIV (Human Immunodeficiency Virus)
Cortical neurons	Viruses- • Alphaviruses • Bunyaviruses • Herpes Simplex Virus (HSV) • Japanese B encephalitis virus (JEV) • Measles • West Nile virus (WNV) • St Louis encephalitis virus • Tick-borne encephalitis virus
Thalamus and Hippocampus	Viruses- • Human Enteroviruses • Rabies virus • West Nile virus
Meninges	Bacteria – • E coli • Listeria monocytogenes • Neisseria meningitides • Guillan-barre syndrome • Haemophilus influenzae b • Mycobacterium Tb • Streptococcus pneumonia • Group B streptococcus Viruses- • Enterovirus • HIV • JEV • Measles • LCMV (Lymphocytic choriomeningitis virus) • Mumps • Nipah virus Fungi- • Cryptococcus neoformans • Histoplasma capsulatum • Blastocystis hominis • Coccidioides immitis
Ependyma	Viruses- • CMV • Enterovirus • Mumps • LCMV
Cerebellum	Viruses-

	• Enterovirus • WNV
Brainstem	Viruses- • Enterovirus • Rabies virus • WNV • Poliovirus
Spinal cord	Bacteria- • Treponema pallidum Viruses- • Ebstein Bar virus(EBV) • CMV • HIV • HTLV-1 • Varicella zoster virus(VZV) • WNV

Flowchart 1:- Basic Approach to diagnostic testing of neurological infection [5]

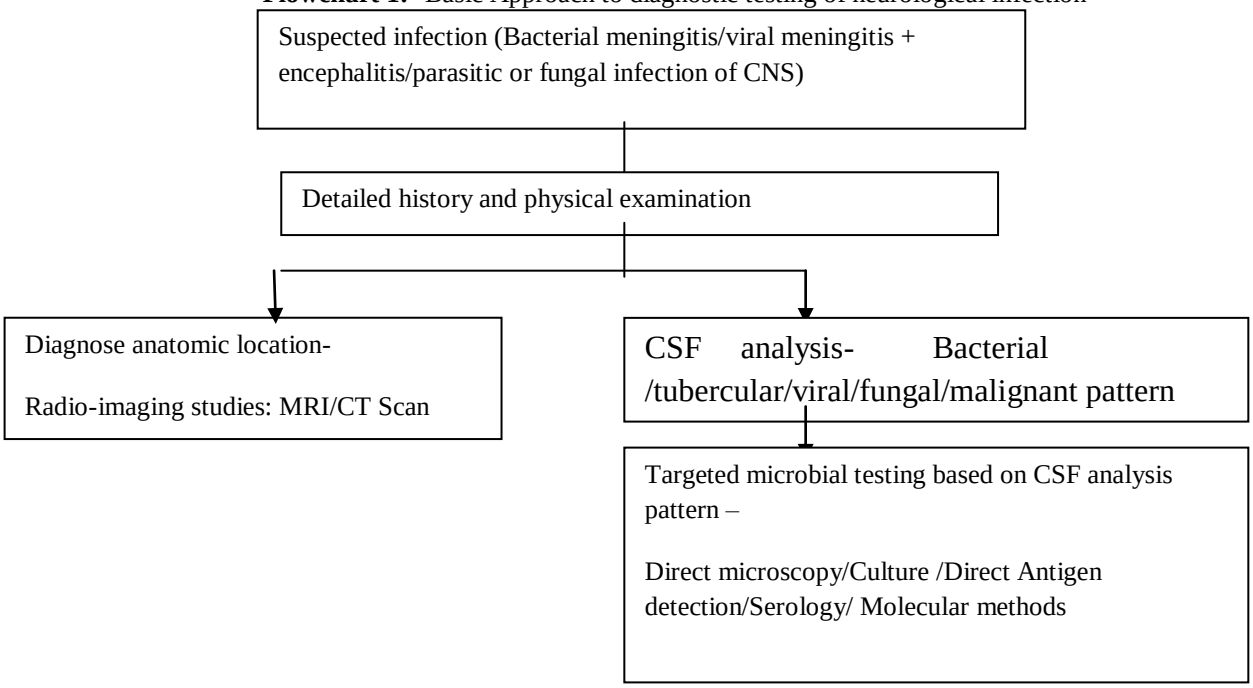

Table 3:- CSF analysis profile [6,7]

Parameter	Normal	Bacterial pattern	Viral pattern	Fungal/malignant pattern	Tubercular pattern
CSF opening pressure	<170mm	Increased	Normal	Normal/increased	>25 cm water
Cell count	<5 cells/mm^3	>1000	<1000	<500	100-500
Predominant cell type	-	Neutrophils	Lymphocytes	Lymphocytes	Lymphocytes
CSF glucose (mg%)	40-70	Decreased	Normal	Decreased	Decreased
CSF protein(mg%)	<45	Increased	Normal	Increased	Increased

Table 4:- Basic laboratory tests available for Targeted microbial testing [5,6,7,8]

Infection type	Microscopy	Culture	Serology	Direct antigen test
Bacterial meningitis				
Sample collection timeline	Before starting empirical therapy; Fever onset	Before starting empirical therapy; First 10 days of Fever/other s/s onset	Acute phase and convalescent phase	Before starting empirical therapy
Sample collection	1 ml CSF by LP; external ventricular drain or shunt fluid	1 ml CSF by LP; external ventricular drain or shunt fluid; Blood (5-10ml)	5ml Blood/serum	CSF 1ml/urine
Container	Sterile disposable container/tube	Sterile disposable container/tube for CSF culture Blood culture bottle(BHI broth) for blood culture	Plain vacutainer	Sterile disposable container
Transport and storage	Immediate transfer to lab at RT; if delay then store at RT or incubator for 24hrs.	Immediate transfer of CSF to lab at RT; if delay then store at RT or incubator for 24hrs.; immediate transfer of blood culture to lab. If delay then refrigerate at 4 ^{o}C. for 24 hrs.	Immediately transfer to lab. If delay then refrigerate at 4^{o}C for 24-48hrs. For longer period store at -20^{o}C.	Immediately transfer to lab. If delay then refrigerate at 4^{o}C for 24-48hrs.
TAT	-2hrs.	2-5 days	6-8 hrs.	1-2hrs
Name of Test	Gram stain; ZN (for AFB)	Routine Bacterial culture and sensitivity; automated MGIT culture/LJ culture for Mycobacteria	Ig M/IgG Antibody detection by ELISA;ICT	Antigen by latex agglutination; ICT
Sensitivity/specificity	60-90%/100%	90%/100%	50-100%	70-100%/100%
Advantage	Rapid; low cost; less TAT	Specific ; Antimicrobial susceptibility testing can be done; Antimicrobial resistance pattern can be studied;MGIT liquid culture medium highly sensitive for Tb bacilli	Better for difficult to culture bacteria.	Rapid; easy to perform; more sensitive than microscopy; no expertise required.
Disadvantage	Low sensitivity; technical expertise required	Contamination may occur; TAT more.	Costly; technical expertise required;cross reactive antibodies may interfere with results.	Costly.
Interpretation	Gives preliminary clue about etiological agent- Gram positive diplococci	Gives confirmatory diagnosis of pyogenic meningitis due to	Useful for seroprevalence and not diagnosis – in	Latex agglutination in CSF for

	suggestive of Streptococcus pneumoniae. Gram negative diplococci suggestive of Neisseria meningitides. Gram negative bacilli suggestive of E coli/Enterobacteriacae. Gram positive bacilli suggestive of Listeria. Gram negative coccobacilli suggestive of Heamophilus influenzae.	common bacterial agents- Streptococcus pneumonia Group B streptococcus E coli/other GNB H influenza Neisseria meningitides(meningococci) Listeria Mycobacterium tb culture positive confirms diagnosis of TBM. Antimicrobial susceptibility pattern can also be reported for specific antimicrobial therapy.	c/o Meningococci Tp antibody by ICT specific test for syphilis, confirms diagnosis of syphilis (all stages). Serological tests not recommended for TBM	capsular antigen confirm diagnosis of – S pneumoniae /N meningitides/E coli/H influenza C-polysaccharide Antigen detection in urine by ICT confirm diagnosis for S pneumoniae.
Viral meningitis /encephalitis Sample collection timeline	Before starting treatment; preferably within 3 days of symptom onset	Before starting treatment; within 10 days of symptom onset	Second week of infection i.e. Acute phase first sample; convalescent phase second sample	Before starting treatment; 5-7dyas of infection
Samples	CSF;fluidaspirate/stool/blood/nasopharyngeal swabs/tissue biopsy/skin scrapings	CSF/fluid aspirate/blood/nasopharyngeal swabs/urine/stool/biopsy/scrapings	Blood /serum	Blood/CSF/
Container	Sterile disposable container/tube with 2-5 ml VTM	Sterile container with 2-5ml VTM	Plain vacutainer	Plain vacutainer
Transport and storage	Sample collected in viral transport medium (VTM) Immediate transfer to lab. At 2-8oC. If delay then freeze at -70oC.	Sample collected in viral transport medium; immediate transfer to lab at 2-8oC. Maintaining cold chain is important. If delay then store at -70oC.	Immediate transfer to lab at RT.if delay then refrigerate at 4oC for 24hrs. for longer storage freeze at -20oC.	Transfer ASAP;if delay then refrigerate.

TAT	24hrs	1-28 days	6-8hrs	2-4 hrs.
Name of test	Test for presence of viral particles/inclusion bodies in clinical sample;	Viral culture(not done routinely; only in reference labs)	IgM/IgG by ELISA; Antibody detection by ICT/HA; IgG avidity ELISA Antigen by ELISA/ICT/HA	Viral antigen by ELISA/ICT /IF assay
Sensitivity/specificity	Low	Low	High	Excellent sensitivity
Advantage	High magnification and resolution,can detect contamination in tissue;	For research use; study antiviral drug resistance;vaccine production;genomic studies	Low cost; mainstay of diagnosis for viral infections; better sensitivity	Low cost;multiple viruses can be detected;less TAT
Disadvantage	Highly expensive; low sensitivity/specificity;detection limit 106 virions/ml./technical expertise required; labor intensive	Labor intensive;expensive;technical expertise required/poor sensitivity and specificity/time consuming	Results depend on disease progression (antibodies not detected in wondow period); technical expertise required;false positive due to cross reactive antibodies.	Result depend on disease progression;
Interpretation	Direct demonstration of virus in clinical sample by electron microscopy confirms diagnosis- Rabies virus/pox virus/adenovirus/ebola virus Direct immunofluorescence microscopy detects virus particles in samples confirms diagnosis- Herpes viruses/adenoviruses/rabies virus Demonstration of inclusion bodies by light microscopy in clinical samples confirms diagnosis- Negri bodies-rabies virus Intranuclear inclusion bodies- HSV/CMV/Poliovirus/adenovirus/Measles virus	Isolation of virus from clinical sample confirms diagnosis- HSV/Rabies virus/measles/pox virus/enteroviruses	Presence of IgM antibody or four fold rise in titre of IgG (in two samples taken one week apart) suggestive of recent infection. Presence of only IgG without rise in titre indicates past or chronic infection. -in measles/Japanese encephalitis/zika virus IgG avidity decreased in primary infection, increased in secondary infection – In measles/ dengue/CMV	Presence of viral antigen confirms diagnosis- NS1 antigen in dengue P24 antigen for HIV CMV antigen Rabies antigen in skin biopsies

Parasitic and Fungal infections of CNS				
Sample collection timeline	Before starting treatment; at symptom onset	Before starting treatment; at symptom onset	Second week of infection	Before starting treatment; 5-7dyas of infection
Samples	CSF(1ml)/Blood(5ml)/skin scraping/tissue biopsy/stool	CSF(10ml)/Blood(5-10ml)/skin scraping/tissue biopsy	Blood/serum/plasma(5ml)- paired sera , one each in acute and convalescent phase	CSF/blood/tissue
Container	Sterile disposable container; for tissue 1ml sterile saline added to the container	Sterile disposable container; for tissue 1ml sterile saline added to the container	Plain vacutainer for blood/serum;EDTA vial for whole blood/plasma	Sterile container
Transport and storage	Immediate transfer to lab at RT. If delay then refrigerate at 4^{o}C.	Immediate transfer to lab at RT. If delay then refrigerate at 4^{o}C.	Immediate transfer to lab at RT. If delay then refrigerate at 4^{o}C.for max 48 hrs. for longer storage freeze at -20^{o}C	Immediate transfer to lab at RT. If delay then refrigerate at 4^{o}C.
TAT	2-4 hrs	1-28days	6-8hrs	2hrs
Name of test	For parasitic infections- Routine stool microscopy for ova/cyst /larva/trophozoites CSF/ tissue for tapeworm hooklets / free living ameobae trophozoites Blood peripheral smear (Giemsa stain) for malarial parasite, tachyzoites of Toxoplasma gondii. Biopsy tissue for tissue cyst (Toxoplasma) For fungal infections – Gram stain for fungal elements India ink examination of CSF for Cryptococcus neoformans. KOH mount for fungal elements Histopathology (H&E stain) of biopsy for fungal elements esp. dimorphic fungi/molds	Parasitic culture not done routinely for diagnosis. Only for reference labs. For fungal infections - Sample for Fungal culture and sensitivity.	For parasitic infection- Ig M /IgG ELISA for toxoplasma/hydatid disease/Taenia inf. For fungal infections- Ig G/IgM/IgAAntibody by ELISA esp for systemic mycoses.	For parasitic infections- Test for malaria antigen by ICT. For fungal infections- Fungal antigen test in clinical sample by Fluorescent antibody staining. Latex agglutination for cryptococcal antigen in CSF. Galactomannan /1,3-beta-D-

				gluan antigen by ELISA for invasive mycoses
Sensitivity/specificity	80-85%/90-100%	Low sensitivity/high specificity	High / low	53-85%/ 85-87%
Advantage	Low cost; easy to perform	Accurate; differentiate recent from past infection; Antifungal testing can be done.	Can be used to asses prognosis; low cost; assist in clinical significance of culture isolates for fungal infections	Significant in early stage of infection; low cost
Disadvantage	Low sensitivity; technical expertise required	It is time consuming/ low yield;	Technical expertise required; Cross-reactive antibodies may occur; false negative in early phase of infection	Poor sensitivity.
Interpretation	Presence of parasite in clinical sample confirms diagnosis. Presence of fungal element gives presumptive diagnosis of fungal infection, however positive histopathology is confirmatory.	Significance of fungal isolate in culture depends on source. Positive culture for dimorphic fungi like Histoplasma and Coccidiodes is confirmatory. For opportunistic/commensal fungi like yeast or Aspergillus spp. – repeat samples should be sent to confirm diagnosis along with clinical correlation and serological tests.	Presence of IgM or Rise in IgG antibody titre between acute and convalescent sera confirms diagnosis- Toxoplasma infection Dimorphic fungi IgG avidity ELISA to differentiate recent from past infection-in Toxoplasmosis. For fungal infections-positive serology confirms diagnosis for systemic mycoses due to dimorphic fungi/ invasive candidiasis.	Positive malarial Antigen by ICT confirms malaria. Positive fungal antigen test confirms diagnosis- Cryptococcal meningitis Invasive Aspergillosis Invasive Candidiasis

Table 5:- Advance laboratory tests for targeted microbial testing [6], [8,9]

Infection type	Name of test	Clinical specimen	Application	TAT	Advantage	Disadvantage
Bacterial meningitis	16s rRNA PCR	CSF	Identify specific pathogens	12-24hrs	Better sensitivity than Culture ;very	Expensive; requires technical expertise and

			by detection of 16s ribosomal RNA		specific	infrastructure
Tubercular meningitis	Nucleic acid amplification tests(singleplex/multiplex)	Blood/CSF / Nasopharyngeal swab/ shunt fluid	RT-PCR and LAMP Assays for particular pathogens- Streptococcus pneumonia Neisseria meningitidis Haemophilus influenza Listeria monocytogenes Streptococcus agalactiae	8-12 hrs	High sensitivity/specificity; useful in patients already on antibiotics	High cost; lab infrastructure required/ technical expertise needed
	MALDI-TOF MS	Blood/CSF	Mass spectrometry identification based on weight	1-2 hrs	Rapid, relatively low cost	Infrastructure requirement;antimicrobial sensitivity cannot be reported.
	GeneXpert	CSF	Cartridge-based PCR	2.5hrs	Rapid, sensitivity similar to culture, highly specific, Easy;	Expensive; limited shelf life of cartridges
	GeneXpert ultra	CSF	Cartridge-based PCR	<2hrs	Quick; better performance ; easy to use	Expensive; Poor negative predictive value
	TRUENAT	CSF	Chip-based micro real time PCR	<2hr	Rapid; easy to use;specific	High cost;poor sensitivity
Viral meningitis/encephalitis	16s rRNA amplification and Nucleic acid amplification tests (singleplex/multiplex)	Blood/CSF / Nasopharyngeal swab/ shunt fluid	Identify specific pathogens by detection of 16s ribosomal RNA and real time PCR assays	24hr	Highly specific	Costly;infrastructure required;technical expertise required

Syndromic approach	BioFire film array ME panel	CSF CSF; Brain tissue	Real time micro PCR detects multiple Pathogens-Neural panel includes – **BACTERIA: Escherichia coli** K1 **Haemophilus influenzae Listeria monocytogenes Neisseria meningitidis Streptococcus agalactiae Streptococcus pneumonia VIRUSES:** Cytomegalovirus (CMV) Enterovirus (EV) Herpes simplex virus 1 (HSV-1) Herpes simplex virus 2 (HSV-2) Human herpesvirus 6 (HHV-6) Human parechoviruses (HPeV) Varicella zoster virus (VZV) **YEAST: Cryptococcus (C.**	2hrs	Rapid; technically less demanding;highly specific 99.8%; 95% sensitivity; single test can identify multiple pathogens	Expensive; can detect only pathogens in the panel

			neoformans/C. gattii)			
	Metagenomic Next-generation Sequencing(mNGS)		Nucleic acid detection of any pathogen-viral and fungal meningitis especially.	12-24 hrs	Highly specific; single test can identify multiple pathogens; can identify new or emerging pathogens.	Costly; Limited availability expensive;technical expertise and infrastructure required; Poor performance seen when indirect tests are required to make the diagnosis (for example, serology), when infections are compartmentalized and for low abundance pathogens.; poor sensitivity if viral load less (<100 copies)

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