BATTLING INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Organisms, Risk Factors, and Laboratory Diagnostics

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Outline

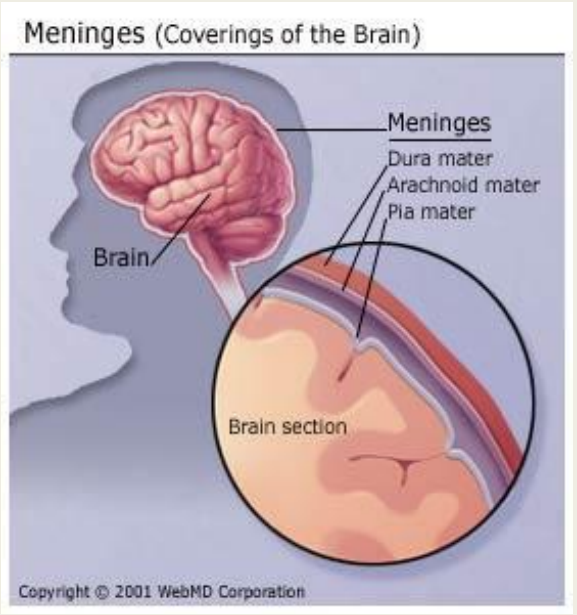
- Overview of meningitis
- Address P.A.C.E. Goals
  - *Identify organisms commonly associated with CNS infection*
  - *Discuss the factors which put patients at risk for these infections*
  - *Explain the strengths and weaknesses of current diagnostic methods*
- Clinical and financial impact of rapid results
- Conclusion

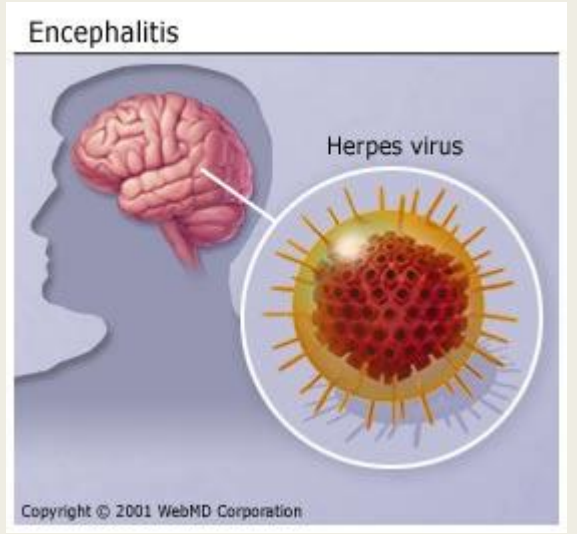
CNS Infections

Meningitis vs. encephalitis

- Meningitis
  - *Infection/ inflammation of meninges (3 layers)*
    - Brain, spinal cord, or intracranial spaces (CSF)
    - Bacterial, viral, fungal

- Encephalitis
  - *Infection/inflammation of brain parenchyma*
    - Infections & non-infectious causes (Injury, cancer, drugs)
    - Diffuse → more typically viral
    - Cerebritis is more focal presentation

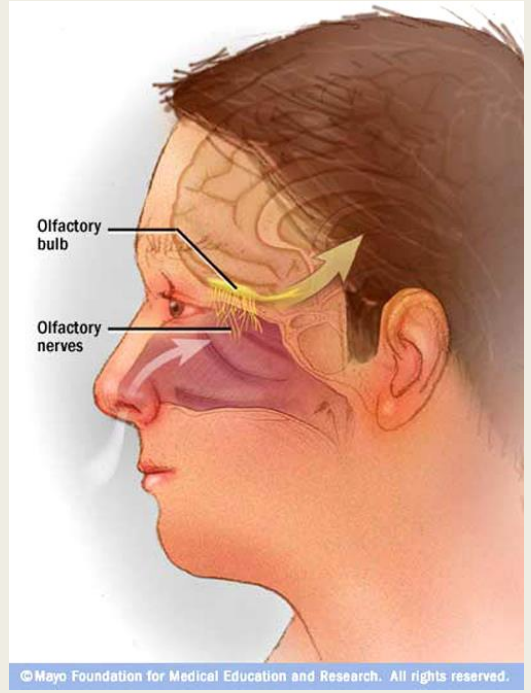


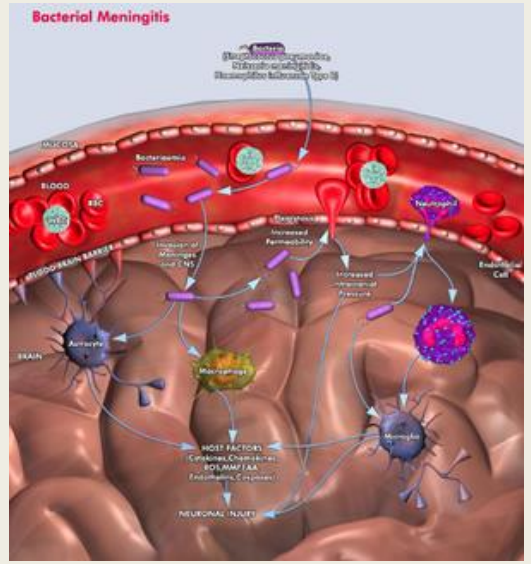


CNS Infections

Routes of infection

- Direct invasion
  - *"Natural" → Access through sinus, conjunctiva*
    - URT flora, amoeba, HSV/GBS (neonates)
  - *"Traumatic" → Open cranial or spinal wound*
    - *Environmental GNR, Skin flora, Mycobacteria, fungi*
  - *"Iatrogenic" → Medical device related, e.g. shunt, drain*
    - *Skin flora*
- Haematogenous
  - *Following infection*
    - Pneumonia, BSI
- Endogenous
  - *Reactivation of latent infection → HSV, CMV, EBV, JC*

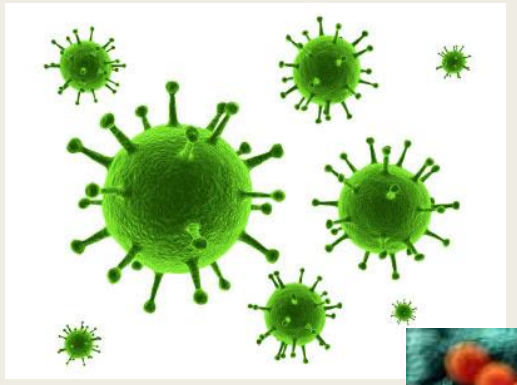


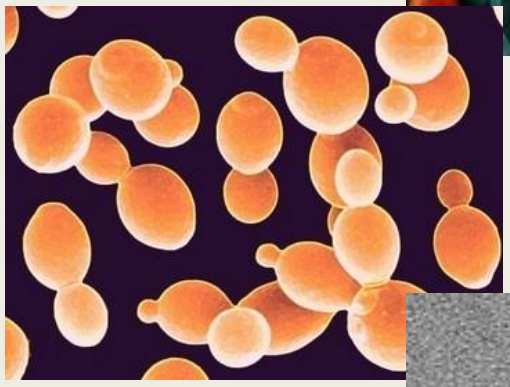


Organisms

Common causes

- Viruses (65-75%)
  - *Commonly self-resolving "aseptic meningitis"*
  - *May be life-threatening in immunecompromised host*
- Bacteria (15-20%)
  - *Severe, acute meningitis*
  - *High mortality if untreated*
- Fungi (5-8%)
  - *Most commonly yeast, dimorphic fungi*
  - *More common in compromised*
- Amoebic (<1%)
  - *Associated with environmental exposure*
  - *Almost uniformly fatal*





CSF characteristics

- General rule
  - *Subject to variation by species, severity of infection, etc.*
  - *Viral may initially have neutrophilic predominance*

Test	Appearance	Pressure	WBC/µL	Protein mg/dL	Glucose mg/dL	Chloride
Normal CSF	Clear	90 – 180 mm	0-8 lymph.	15-45	50-80	115-130 mEq/L
Acute bacterial meningitis	Turbid	Increased	1000 -10000	100 – 500	< 40	Decreased
Viral meningitis	Clear	Normal to moderate increase	5-300, rarely >1000	Normal to mild increased	Normal	Normal
Fungal meningitis	Clear	Increased	40-400 mixed	50-300	Decreased	Decreased

Risk factors

- Age
  - *Neonates*
    - Congenital infection → CMV, HSV
    - Vertical transmission during birthing → HSV, GBS
  - *Young children*
    - High rate of URT colonization → *S. pneumoniae, H. influenzae, N. meningitidis,*
    - Questionable hygiene → Enterovirus
- Immune state
  - *Compromised → HIV/AIDS, HSCT, SOT*
    - Typically more severe in compromised
    - Often re-activation of latent infection (VZV, HSV, JCV); fungal infections
- Medical hardware
  - *CNS shunts/drains/catheters*
    - Skin flora, GNRs → biofilm

BACTERIAL MENINGITIS

Clinical impact of bacterial meningitis

- **Acute bacterial meningitis is life-threatening condition (i.e. *critical value!*)**
  - *Critical role for Laboratory*
    - Differentiate from viral meningitis (more common/less severe)
    - Benefit from prompt abx

- **General risk factors**
  - *Age, colonization status, indwelling devices*

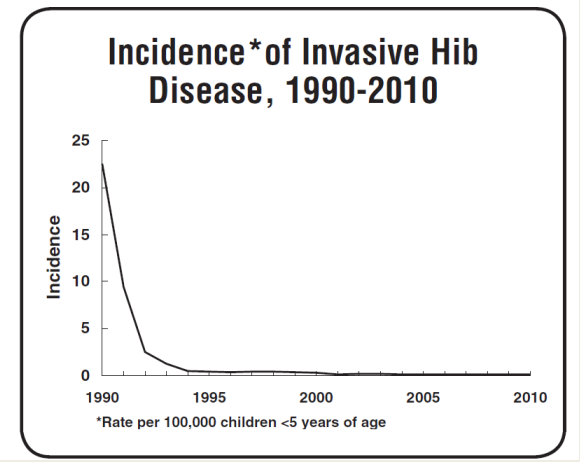
Demographic	Common Bacterial Etiology
Neonate	*S. agalactiae, E. coli, L. monocytogenes*
Infants, young children	*H. influenzae, S. pneumoniae, N. meningitidis*
Young adult	*N. meningitidis*
Adult	*S. pneumoniae, N. meningitidis*
Elderly	*S. pneumoniae, L. monocytogenes, Enterobacteriaceae, NLFs*
CNS shunt/drain	*CoNS, S. aureus, Corynebacterium spp., Enterobacteriaceae, NLFs*

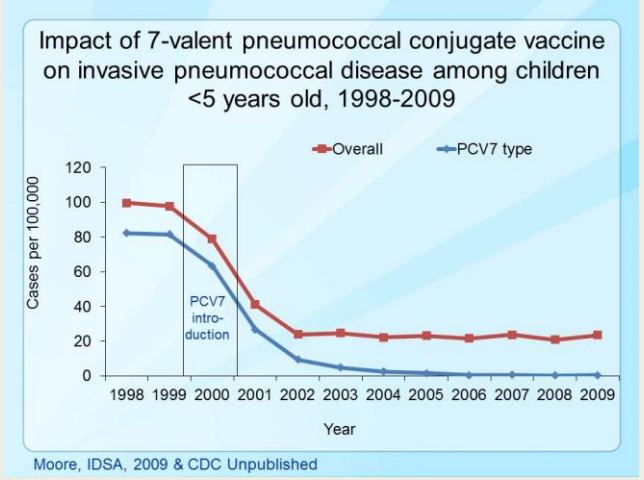
Epidemiology

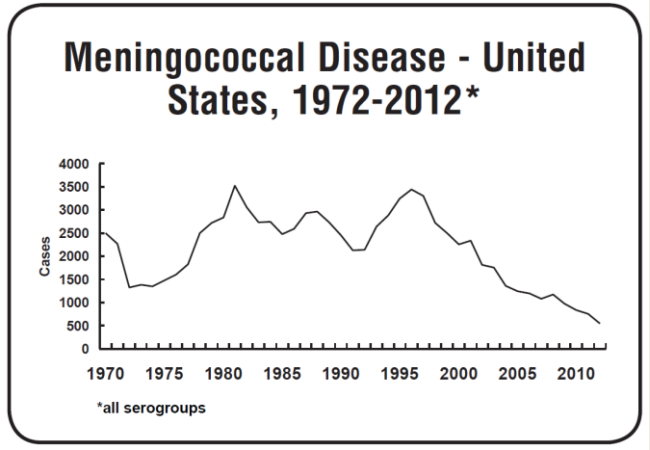
- Initial surveys in early 1980's
  - *Attack rate of 3.0-6.0 cases/100,000*
    - 10-20x higher for children <1 yoa
  - *Common agents…source of infection?*
    - 75-85% → *H. influenzae, S. pneumoniae, N. meningitidis*
    - *2-5% → S. agalactiae (neonates, now elderly as well) L. monocytogenes*
    - 2-5% → *Enterobacteriaceae, Staph spp., Strep spp., P. aeruginosa*
- Vaccine impact?

Epidemiology

- **Vaccine impact?**
  - *Dramatic reduction, changing epidemiology*
    - HiB conjugate (1990) → >99% reduction from 54 to 0.3 cs/100k
    - Pneumococcus (2000)
      - *Pediatric conjugate 7/13: 97% effective, 30-60% decrease in pneumococcal meningitis*
      - *Adult polysaccharide 23: High risk adults → includes 75-90% of CSF isolates*
    - *N. meningitidis* ACYW (2005) B (2015) → 65% reduction from 0.92 to 0.33 cs/100k
      - *Not recommended for general population in USA (low risk)*
      - *Recommended for laboratory workers (60-100x higher incidence than general public), college freshmen*

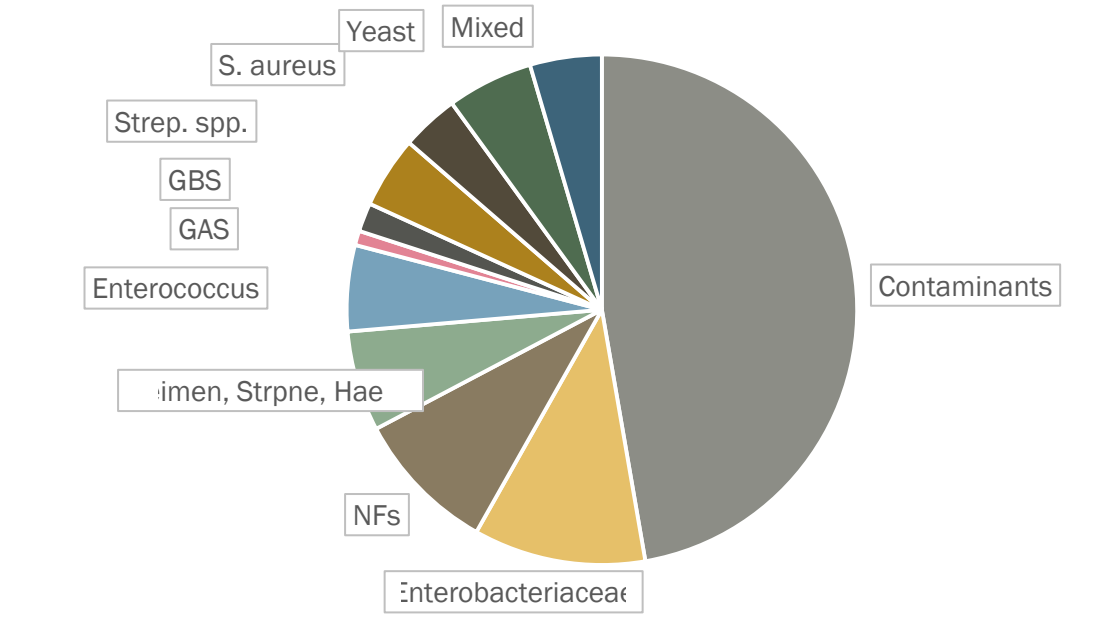






Epidemiology

- **Current causes of bacterial meningitis**
  - *WDL (2 years)*
    - *CoNs leading – CSF shunts*
      - *real vs. contaminant!*
    - *Broth or plate only? Single CFU? 1st quadrant?*

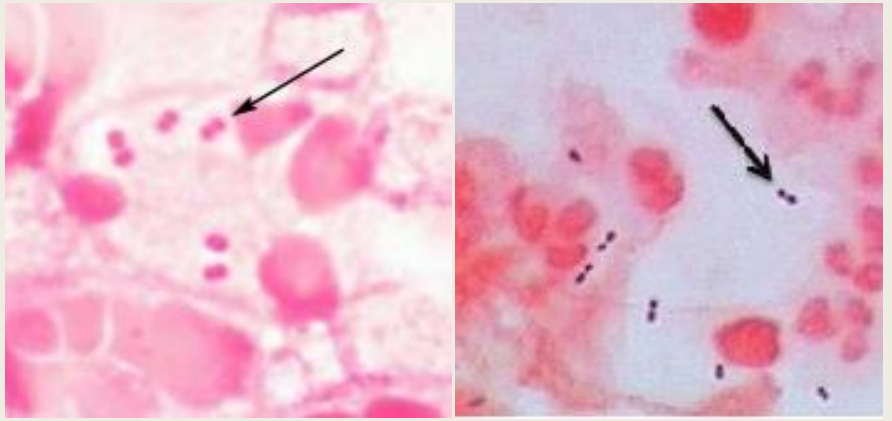


ID	# cultures	% cultures
S. epidermidis	39	35.5%
CoNS	5	4.5%
Corynebacterium	1	0.9%
P. acnes	4	3.6%
Bacillus spp.	2	1.8%
Micrococcus	1	0.9%
S. pneumoniae	4	3.6%
*N. meningitidis**	3	2.7%
E. coli	4	3.6%
S. marcessens	3	2.7%
Enterococcus	6	5.5%
P. aeruginosa	7	6.4%
Enterobacter spp.	4	3.6%
S. agalactiae	2	1.8%
S. pyogenes	1	0.9%
Acinetobacter	3	2.7%
Viridans gr. Strep.	5	4.5%
S. aureus	4	3.6%
P. mirabilis	1	0.9%
Mixed pathogens	5	4.5%
Candida spp.	2	1.8%
C. neoformans	4	3.6%
Total	110	

Detection methods

- Direct exam
- Antigen
- Culture
- NAAT/PCR

Detection methods

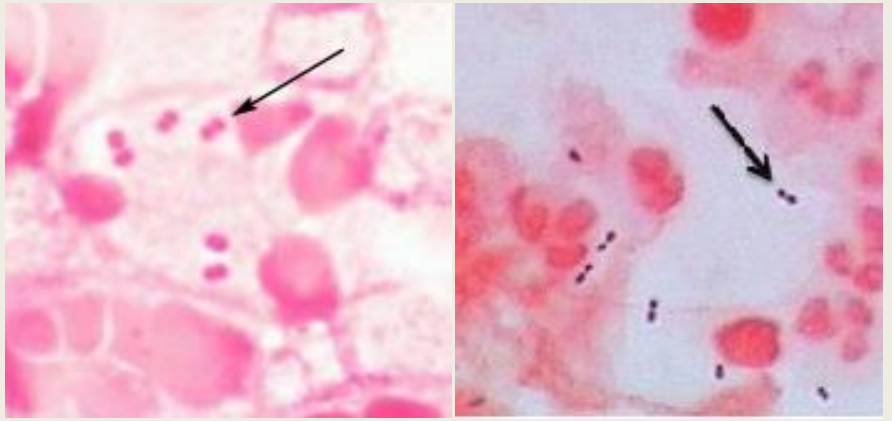


Direct exam

- Critical value!
  - *Critical value → establish acceptable TAT for reporting (<2 h)*
    - Cellularity (RBCs, PMN vs. Monos)
    - Bacteria (presence, relative abundance, morph, location)

- Sensitivity?

Detection methods



Direct exam

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  - *Critical value → establish acceptable TAT for reporting (<2 h)*
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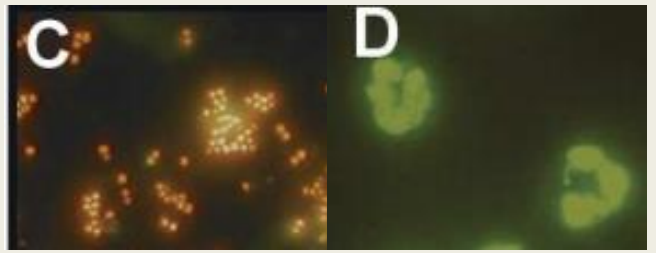
    - Bacteria (presence, relative abundance, morph, location)

- Sensitivity? *Concentrate!*
  - *Stains*
    - Gram stain → Morphology, GP/GN
      - *Variable sensitivity (LoD ~ 10^6 cfu/mL), small GNR*
      - *Specificity for "rare GPC"*
    - Acridine Orange → Morphology only
      - *Fluorescent nucleic acid stain*
      - *Increased sensitivity (LoD ~ 10^4 cfu/mL)*
      - *Sensitivity -"Rare GNR"; Specificity -"Rare GPC"*

Pathogen	Sensitivity (%)[a]
	CSF Gram stain
Haemophilus influenzae	25–65
Streptococcus pneumoniae	69–93
Neisseria meningitidis	30–89
Listeria monocytogenes	10–35
Streptococcus agalactiae	80–90
Streptococcus pyogenes	66–73
Streptococcus suis	50
Staphylococcus aureus	20–44

CLINICAL MICROBIOLOGY REVIEWS, July 2010, p. 467–492

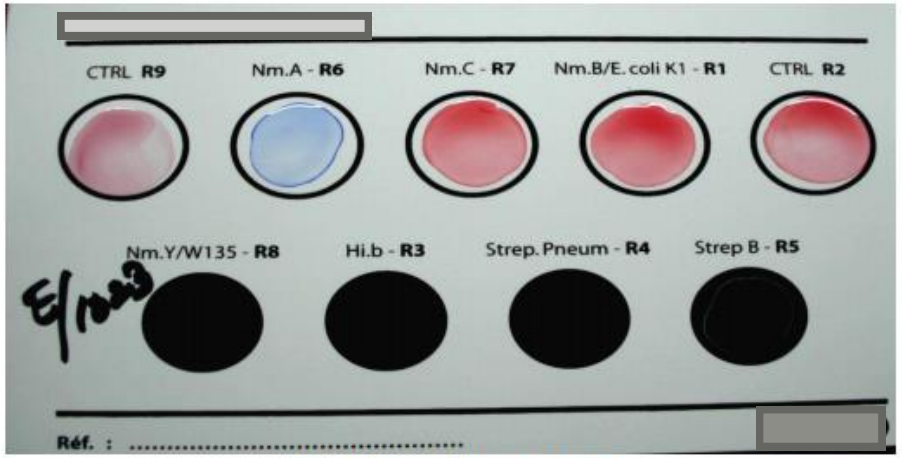


Journal of Medical Microbiology (2005), 54, 843–850

Detection methods

Antigen

- Types
  - *Latex agglutination, Enzyme assay*
    - *N. meningitidis*, HiB, *S. pneumoniae, GBS*
    - Simple, faster than culture (10-20 min.)
- Sensitivity?
  - *vs. Gram stain? Non-viable organisms? Abx?*



Neisseria meningitides A – Positive
Group B Streptococci – positive

Detection methods

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    - Sensitivity similar or inferior to Gram stain
    - No added sensitivity for patients on Abx
    - Potential for "false sense of security" w/ neg result
      - *"No substantial benefit beyond concentrated Gram stain"*

918 CSF specimens
38 Culture (+)
4 GS (+), Culture (-)

Gram stain result	No. (%) of specimens	
	BAT positive	BAT negative
Positive	26 (62)	11 (26)
Negative	3 (7)[a]	2 (5)

Specimens with organisms not detected by BAT and Gram stain

Organism	Total no. of specimens	No. (%) of specimens not detected by:	
		BAT	Gram stain
Detected by culture[a]			
Streptococcus pneumoniae	22	6 (27)	3 (14)
Group B *Streptococcus* species	7	3 (43)	0 (0)
Neisseria meningitidis	7	3 (43)	1 (14)
Haemophilus influenzae type b	2	0 (0)	1 (50)
Not detected by culture[b]	4	1 (25)	0 (0)
Total	42	13 (31)	5 (12)

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2010, p. 1504–1505

Detection methods

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- MIC.22550 – Back-up cultures required on <u>both</u> AG-positive and negative CSF specimens

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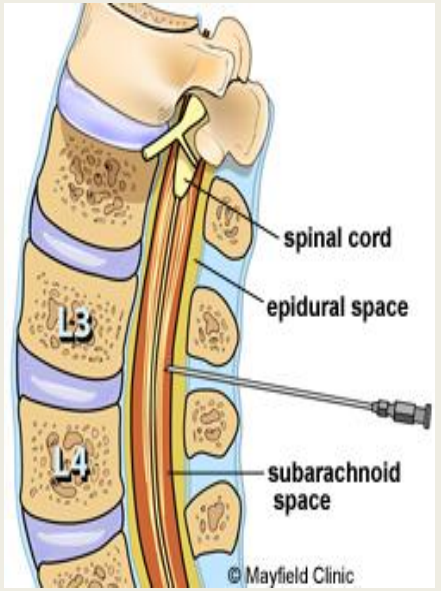
Detection methods

Culture

- "Gold standard"
  - *Tube #2 → Centrifuge or plate entire volume (<1 mL)*
    - Aerobic culture (BAP, CHOC) + Thio broth
- Sensitivity?



Tube 1 – Chemistry (Glucose, Protein)
Tube 2 – Microbiology (GS, Culture)
Tube 3 – Hematology (Cell count, Dif)

Detection methods

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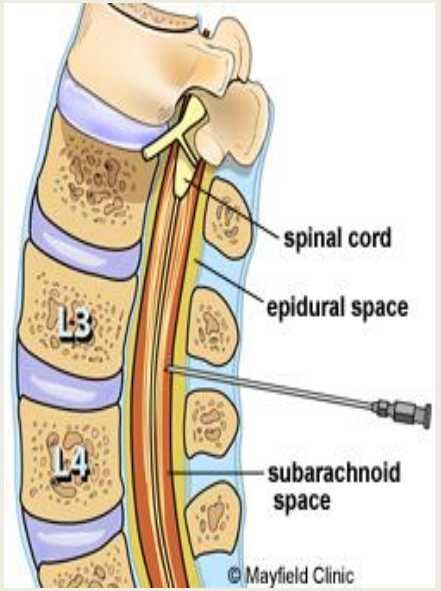
- Sensitivity?
  - *80-95%*
  - *Factors impacting culture sensitivity*
    - Organisim: 95% *H. flu*, 90% *S. pneumo*, 80% *N. mening*
    - CSF volume: some infections ≤10^3 CFU/mL
    - Abx usage: 60-80% *decrease* in sensitivity
    - Gold standard: Cytology (>1000 WBC/uL, >80% PMN)
- Blood culture added benefit?



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Culture Sensitivity vs. Duration of Abx

None	< 4h	> 4h	>12 h	>24 h
84% (146/159)	72% (18/25)	55% (26/47)	58% (19/33)	59% (17/29)

PEDIATRICS Volume 122, Number 4, October 2008

CLINICAL MICROBIOLOGY REVIEWS, July 2010, p. 467–492

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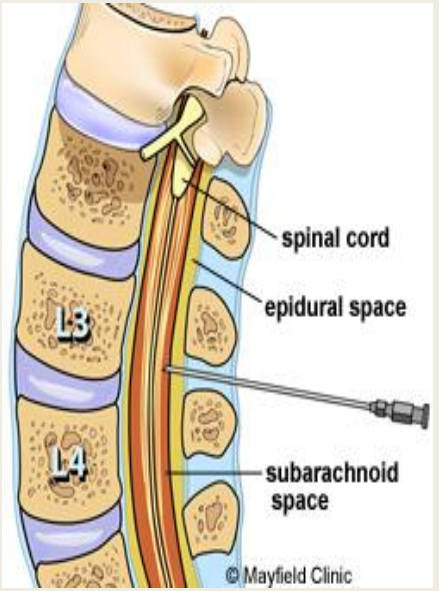

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PEDIATRICS Volume 122, Number 4, October 2008

Sensitivity of GS, BC, CSF Culture

Positive CSF Gram-stain results[a]	95/150 (63)
Positive blood culture results[b]	123/187 (66)
Positive CSF culture results[c]	136/154 (88)

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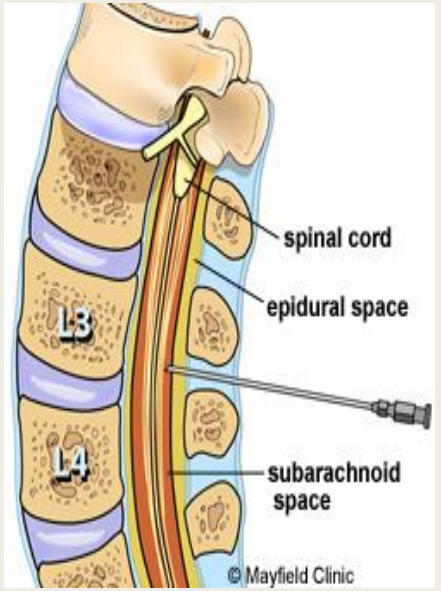

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Staphylococcus aureus	75–100	20–44

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Detection methods

NAAT/PCR

- Potential advantages
  - *Speed, sensitivity, less impact of abx treatment*
    - Highly desirable for *N. meningitidis*
      - *Lowest GS and culture sensitivity among bacterial pathogens*
      - *Rapidly progressing and fatal*
- Performance
  - *Sensitivity: 90-97%; Specificity: >99%*
  - *Result available in 2-3 h*
  - *~20% decrease in sensitivity if abx*

Gram staining, culture, and PCR of CSF for diagnosis of meningococcal meningitis in 38 infected patients

Test	No. of samples		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Gram staining	25	13	66	100
Culture	21	17	55	100
Gram staining culture	33	5	87	100
PCR	37	1	97	99.6

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2003, p. 3851–3853

Clinical presentation	PCR-positive[a]		LA-positive[a]		Culture-positive[a]	
	CSF	Blood	CSF	Blood	CSF	Blood
Meningitis	27 (100)	11 (40)	12 (57)	9 (43)	12 (43)	11 (41)

Table 2. Influence of antibiotic therapy on the result by diagnostic method in blood and cerebrospinal fluid (CSF)

	Test[a]	Tests done	Positive (%)	p value
Collection of CSF samples				
Before onset of antibiotic treatment	PCR	21	21 (100)	
	LA	15	9 (60)	
	Culture	23	12 (52)	
After onset of antibiotic treatment	PCR	16	13 (81)	0.07
	LA	9	2 (22)	0.10
	Culture	14	1 (7)	0.01

Clin Microbiol Infect 2006; 12: 137–141

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  - *Result available in 2-3 h*
  - *~20% decrease in sensitivity if abx*
- PCR considered "gold standard" for *N. meningitidis* in UK
  - *Observed a 56% increase in lab-confirmed meningococcal disease*

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	Culture	14	1 (7)	0.01

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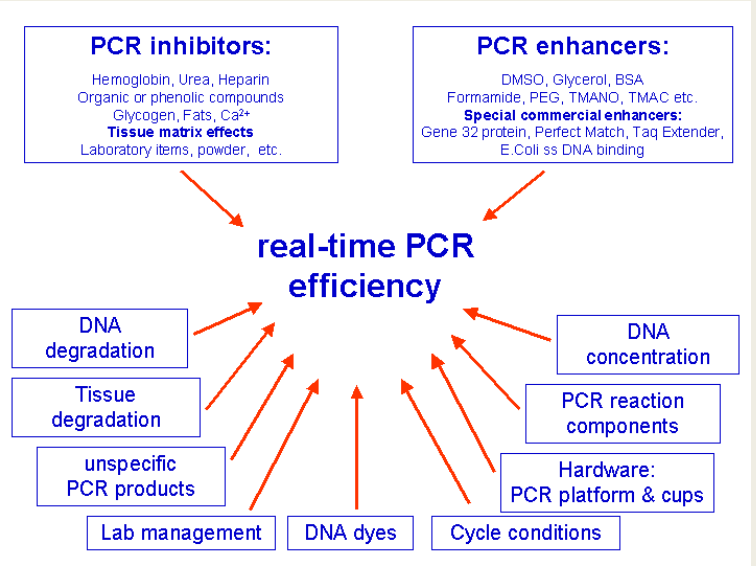
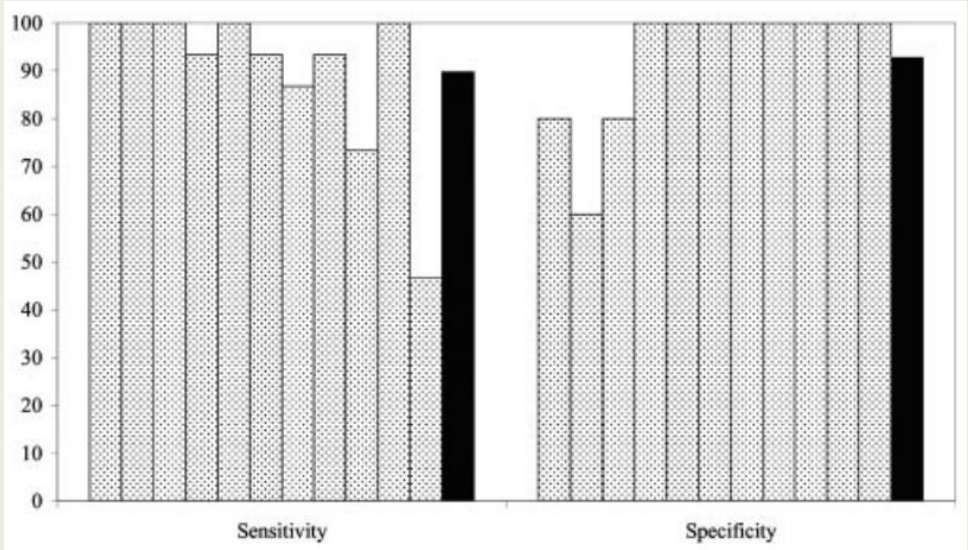
Detection methods

NAAT/PCR

- Obstacles to NAAT
  - *Few FDA-cleared options → LDT?*
    - Complex to design
      - *<u>Inhibitors</u> - Elevated proteins, globulin, cellular infiltrates, hemin*
      - *<u>Targets</u> – Binding affinity/strain diversity*
    - Lab/lab variability
    - Lack of clinical samples to validate
    - Singleplex lacks broad applicability
      - *indistinguishable clinical presentation among bacterial (and sometimes viral) meningitis cases*



Interlaboratory comparison of *N. meningitidis* NAAT

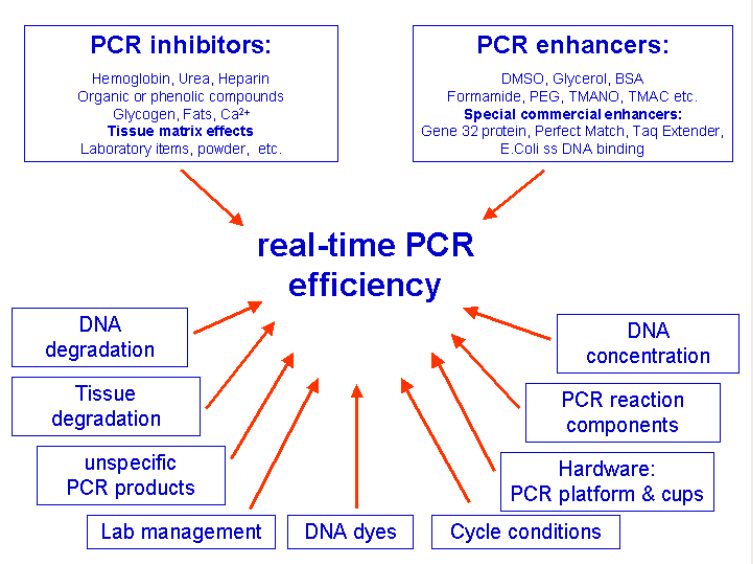


JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 144–149

Detection methods

NAAT/PCR

- Obstacles to NAAT
  - *Few FDA-cleared options → LDT?*
    - Complex to design
      - *<u>Inhibitors</u> - Elevated proteins, globulin, cellular infiltrates, hemin*
      - *<u>Targets</u> – Binding affinity/strain diversity*
    - Lab/lab variability
    - Lack of clinical samples to validate
    - Singleplex lacks broad applicability
      - *indistinguishable clinical presentation among bacterial (and sometimes viral) meningitis cases*
  - *Multiplex?*
    - More complex
      - *Annealing temps., bacterial v. bacterial + viral?*



Organism	PCR-positive patients: Culture confirmed	Probable	Sensitivity
Neisseria meningitidis	25/27 (92.6%)	6/6 (100%)	93.9% (86.8–93.9)
Streptococcus pneumoniae	17/18 (94.4%)	7/8 (87.5%)	92.3% (83.4–92.3)
Haemophilus influenzae type b	7/8 (87.5%)	0 (0)	88% (53.9–88)

Clin Microbiol Infect 2005; 11: 386–390

VIRAL MENINGITIS

Clinical impact of viral meningitis

- **“Aseptic meningitis”**
  - *Mild/self-resolving to acute and life-threatening*
  - *~35,000 hospitalizations/yr → 14/100,000*
    - Critical role for Laboratory
      - *Differentiate bacterial meningitis (less common/more severe) and severe viral etiologies*
      - *Management: Antiviral Rx? Supportive therapy?*

- **General risk factors**
  - *Age*
  - *Immunecompromise (HIV or suppressive therapies)*
  - *Exposure*
    - Outdoor activities, geographic location, season
      - *Endemic areas for virus/vector*
      - *Outdoor activities*
      - *Community - pools, daycare*

Epidemiology

- **Prevalence**
  - *Viral etiologies are the most common causes of meningitis (70-80%)*
    - 50-70% of “aseptic meningitis” go without specific diagnosis/viral ID
    - Demographic most affected depends on specific virus
- **Common agents…source of infection?**
  - *Enteroviruses*
    - > 10 million cases/yr in US → direct person-person spread (feces, saliva, fomites, water)
  - *Arboviruses*
    - *~100-200k infections/yr in US, ~1% severe symptoms → Arthropod-borne (mosquito, tick)*
  - *Herpesviruses*
    - *Recurrent meningitis in young adults, severe infection in compromised host → reactivation*
  - *Polyomaviruses*
    - *Exclusively compromised host, 1-8% of HIV patients pre-HAART → reactivation*
- **Vaccine impact?**

Detection methods

- Culture
- Serology
- NAAT/PCR

Best method depends on…

specific virus, time from onset of symptoms, available tests, specimen

Detection methods

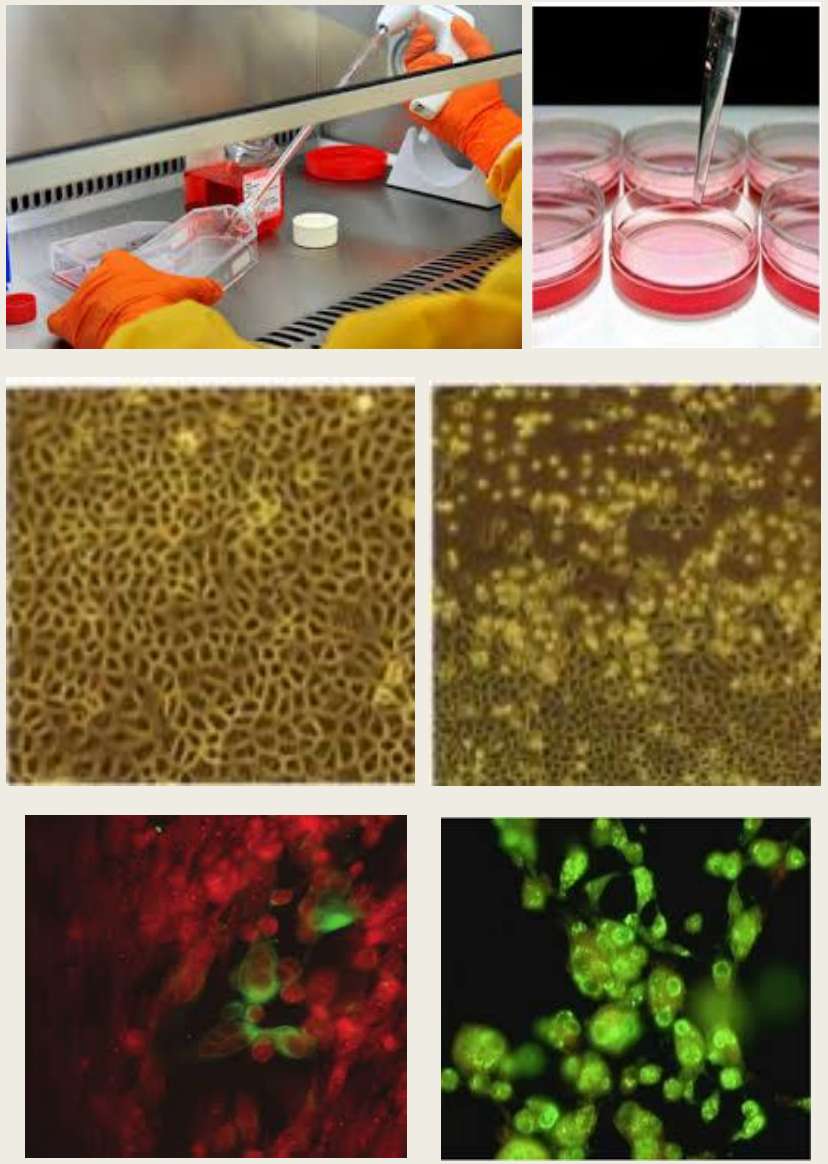
Virus	Effectiveness[b] of diagnosis by:							
	PCR		Serology[c]		Culture of specimens			
	Serum	CSF	Serum	CSF	Throat	Rectal	Blood	CSF
Arboviruses	+[d]	+[d]	++	++	−	−	++	+/−
WNV	+[d]	+[d]	++	++	−	−	−	−
Enteroviruses								
Nonpoliovirus	+	++	+	−	+	++	+/−	++
Poliovirus	+	++	+[e]	−	+	++	−	−
Herpesviruses								
CMV	+	++	+	++	+	−	+	Rare
EBV	+	+	++	++	+/−	−	+/−	+/−
HHV-6	+/−	+/−	+/−	−	−	−	+[d]	+[d]
HSV-1, and HSV-2	−	++	+/−	+	−	−	−	−
VZV	++	+	+[e]	++	−	−	−	+
JCV	−	++	−	−	−	−	−	−

CLINICAL MICROBIOLOGY REVIEWS, Oct. 2004, p. 903–925

Detection methods

Culture

- Traditional “Gold standard”
  - *Prepare monolayer of permissive cells (CAP MIC.61180)*
    - Green monkey kidney, MRC-5, A549, MDCK
    - Seed to microwell plate or culture tube
  - *Inoculate w/ specimen*
    - Antibiotics – non-sterile site, lab contamination
    - Incubate depending on virus
  - *Observe for CPE*
    - On regular schedule, typically every 48-72
      - *Cell lysis, vacuolization, syncytia, inclusion bodies*
      - *Semi-specific*
  - *Stain w/ panel of virus specific Abs*
    - Final ID



Detection methods

Culture - Disadvantages

- Poorly sensitive
  - *CSF culture yield especially low, not typically recommended for diagnosis*
    - HSV ~ 20% sensitive, EVs 30-35% sensitive, JC not cultivable using standard cell lines
  - *Potentially due to presence of neutralizing Ab, low VL in CSF*
    - Preventing uptake of viruses by host cells

- Extended TAT
  - *Growth rate of some viruses e.g. VZV, can take up to 14-28 days* (CAP MIC.61210)
    - Limits clinical utility for diagnosis

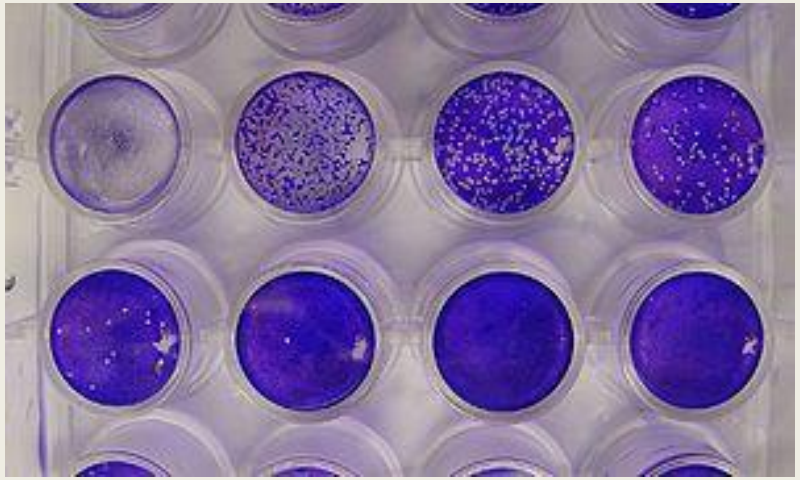
- Technical aspects of culture
  - *Maintaining multiple cell lines*
  - *Contamination*
  - *Maintain proficiency of personnel*

Detection methods

Culture - Advantages

- High specificity
  - *Growth indicates viable virus, infectious etiology*
- Broad inclusivity
  - *Not limited by design of PCR target, availability of specific Ab*
    - Discovery of novel viruses
- Increased specificity
  - *Plaque-reduction neutralization assay (PRNT)*
    - Add virus + dilutions of specific AB to each row
    - Determine 50% reduction from no ab control
    - Differentiate b/w closely related viruses (e.g. *flaviviridae*)
- Epidemiology
- Antiviral resistance testing

Increasing Ab concentration



PRNT

Enteroviruses

- Virus
  - *Picornaviridae (enterovirus, echovirus, coxsackievirus)*
    - Non-enveloped >60 serotypes
- Epidemiology
  - *Summer-fall, primarily in children <5 yoa*
    - Transmitted in feces, saliva, environmental sources (water)
  - *80-90% of aseptic meningitis when etiology is found*
- Symptoms
  - *Largely asymptomatic or sub-clinical*
    - Non-specific rash, fever, headache, URT symptoms, etc.
  - *<5% Progress to more severe symptoms*
    - Severe meningitis/encephalitis, Guillian-Barre syndrome
    - Cause severe sepsis syndrome in newborns
      - *Pleocytosis not significantly differet from controls in <1mo*

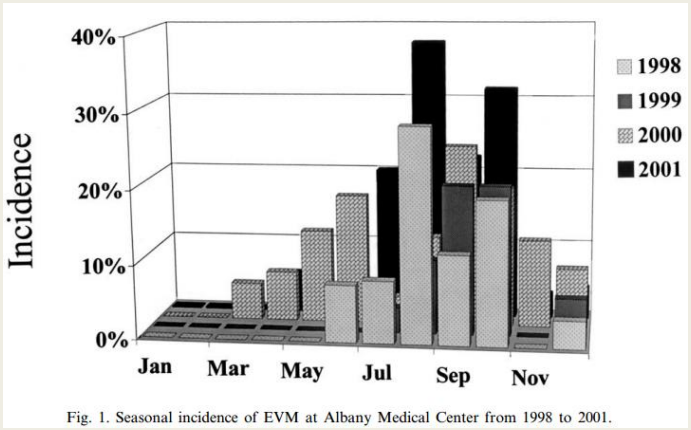


Fig. 1. Seasonal incidence of EVM at Albany Medical Center from 1998 to 2001.

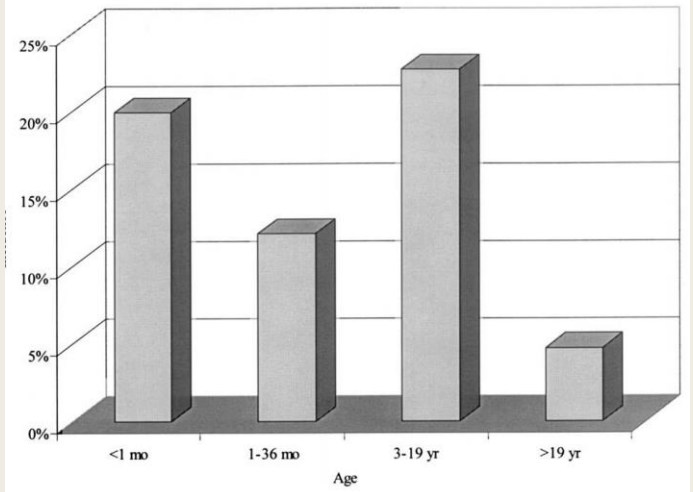
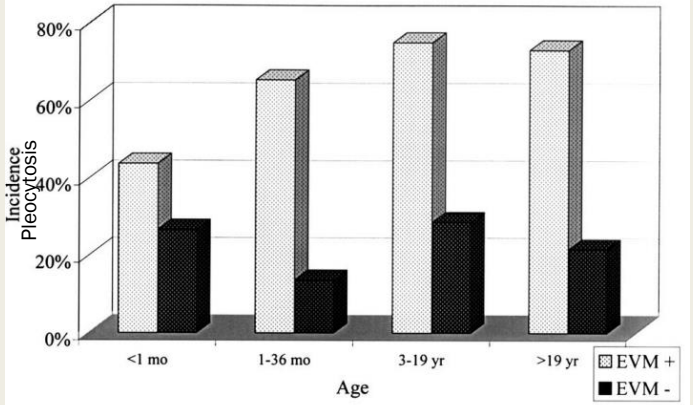


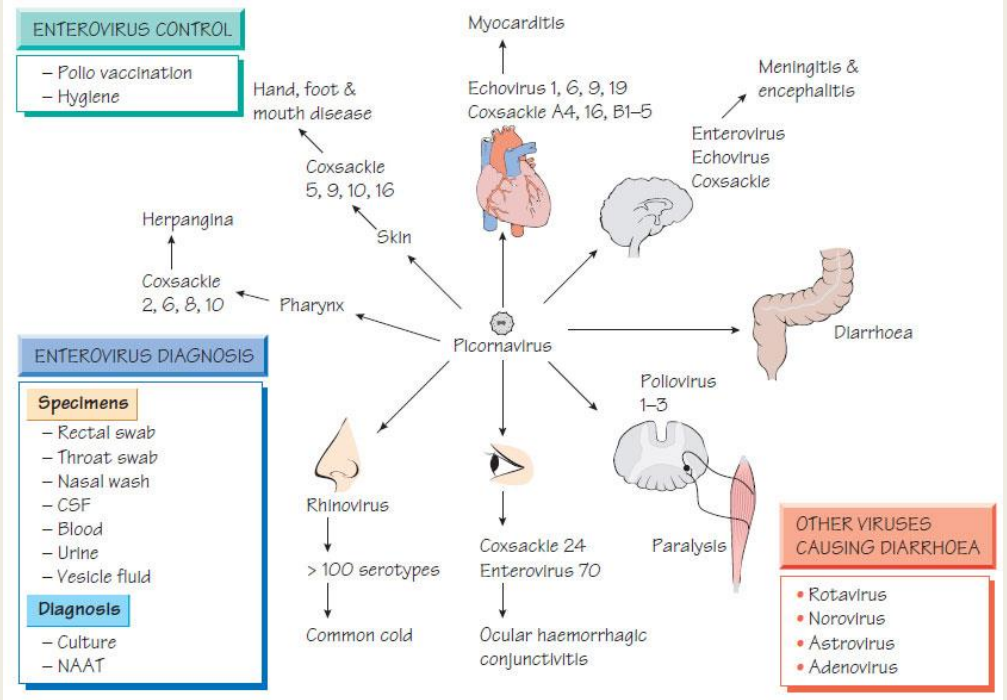
Fig. 2. Incidence rates of EVM by age.



Enteroviruses

Diagnostics

- Culture
  - *CSF → Insensitive (20-60%), slow (5-8 days)*
  - *Resp/GI → non-specific (shed in stool, resp for 4-16 weeks)*
- Serology
  - *Non-specific, high rate of seropositivity*
  - *Many serotypes complicate diagnosis*
- NAAT
  - *Fast → <24 h*
  - *Comprehensive → 5'UTR target encompasses all serotypes*
  - *Sensitive → 10^2-10^3 copies/mL*
  - *Surrogate less invasive specimens…CSF vs. Blood?*
    - Viremia in only 40-60% of CSF (+) patients → not rule out EV meningitis
    - EVs shed in resp, GI…NAATs may cross-react with rhinovirus



http://www.biocyclopedia.com/index/medicinal_microbiology/images/38_large.jpg

Xpert EV (2009)

- Xpert EV (Cepheid)
  - *Qualitative detection of >60 EV serotypes → 140 ul CSF, 2.5 h TAT*
    - coxsackievirus, echovirus, and enterovirus

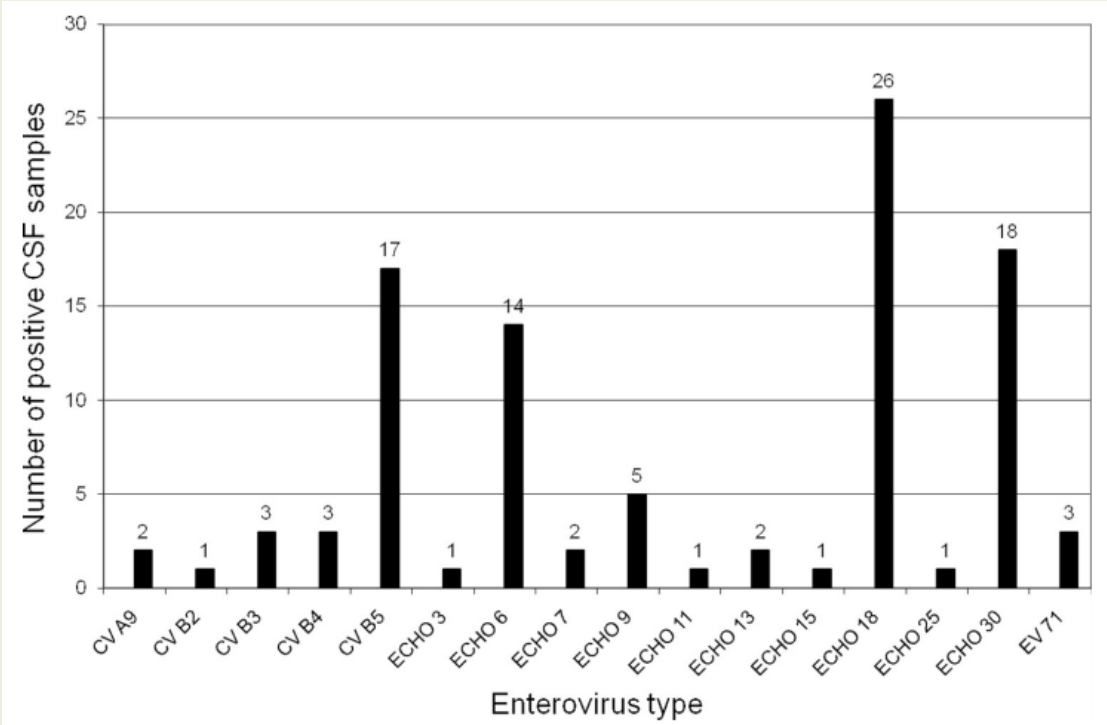
Xpert EV (2009)

- **Performance**
  - *Multicenter*
    - 199 prospective, 235 retrospective
    - Compared to LDTs and culture
  - *Sensitivity: 95%, specificity: 100%*
    - Culture positive in only 35% of specimens

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2011, p. 528–533

TABLE 1. Xpert EV assay for diagnosis of enteroviral meningitis (n = 434)

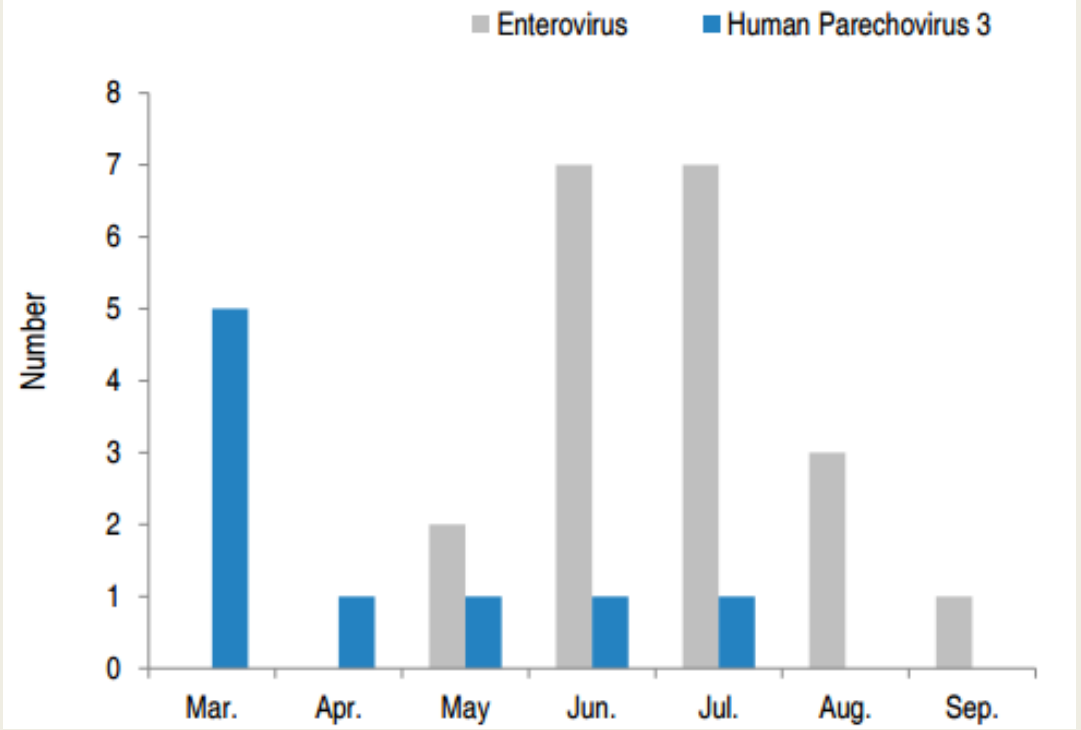
Site	No. of samples[a]					% [b]	
	Total	TP	FP	TN	FN	Sensitivity	Specificity
1	15	2	0	13	0	100	100
2	34	8	0	24	2	80	100
3	44	3	0	41	0	100	100
4	84	27	0	57	0	100	100
5	22	6	0	16	0	100	100
6	235	61	0	170	4	93.85	100
Total	434	107	0	321	6	94.69	100



Xpert EV (2009)

- **Performance**
  - *Multicenter*
    - 199 prospective, 235 retrospective
    - Compared to LDTs and culture
  - *Sensitivity: 95%, specificity: 100%*
    - Culture positive in only 35% of specimens
- **Drawbacks**
  - *May suffer cross-reactivity w/ Rhinovirus*
    - …but this should not be in CSF
  - *Does not include hPeV*
    - 20-30% of “enteroviral aseptic meningitis”
    - Indistinguishable symptoms
    - Similar seasonality (summer-fall)

Korean J Pediatr 2015;58(3):102-107



	Walters 2011	Sharpe 2013	Han 2013	Seo 2015
Location	USA	USA	Korea	Korea
EV	8.3%	14.0%	21.3%	7.5%
HPeV 3	2.4%	17.0%	6.5%	3.4%

Majority of patients < 5 years of age

Xpert EV (2009)

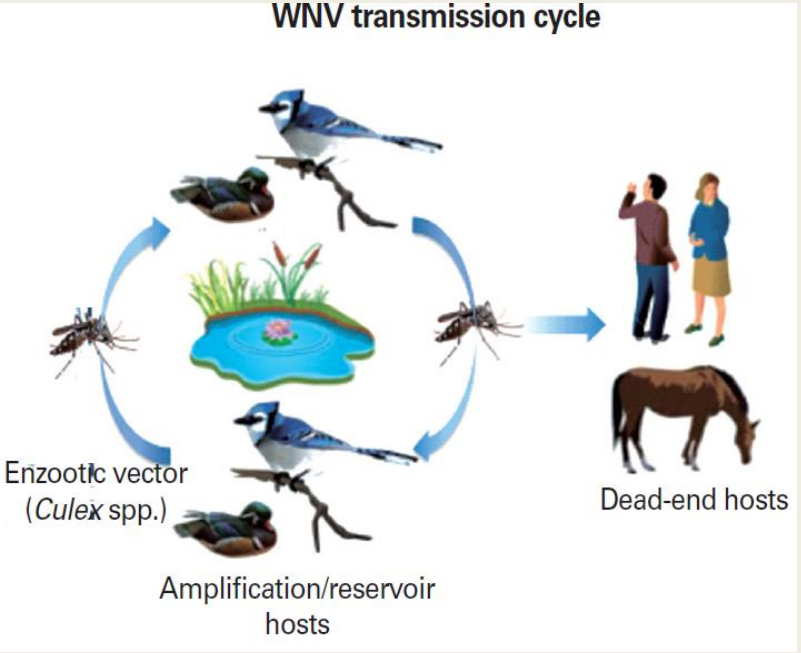
- **Clinical Impact**
  - *EV leading cause of meningitis in children < 5 yoa*
    - Rapid result, suggests non-bacterial meningitis
  - *50 children presenting with meningitis symptoms, EV positive*
    - If EV positive result reported in < 24 h
      - *Abx usage reduced by ~20 h*
      - *Hospital charges reduced by ~$2,800*

Variable	Time from Specimen Collection to Positive PCR Report			
	≤24 h (n = 17)	>24 h (n = 33)	Difference	P
Antibiotic duration				
Mean (h)	22.6	42.2	19.6	0.006
Range	0–48	0–106		
Hospital charges				
Mean	$3035	$5833	$2798	0.001
Range	$891–5937	$1406–16 761		

Arboviruses

- Virus
  - *Diverse group of viruses transmitted by arthropods*
    - *Togaviridae – Eastern Equine, Western Equine, Venezuelan Equine*
    - *Bunyaviridae – La Crosse, Jamestown Canyon, California encephalitis*
    - *Flaviviridae – West Nile, St. Louis, Powassan, Tickborne encephalitis*
  - *Epidemiology*
    - Largely driven by season/climate/vector range
    - Reservoir (amplifying host)
  - *Likely underreported, >99% asymptomatic*

- Symptoms
  - *Fever, rash → meningitis, encephalitis, flaccid paralysis*
    - Attack rate, severity of symptoms highly variable
  - *Hemorrhagic fever groups*
    - Dengue, YFV, Rift Valley, Crimean-congo



Clin Exp Vaccine Res 2014;3:58-77

Arboviruses – USA, 2014

2014. MMWR Morb Mortal Wkly Rep 2015;64:929-34

	Virus type											
	West Nile (N = 2,205)		La Crosse (N = 80)		Jamestown Canyon (N = 11)		St. Louis encephalitis (N = 10)		Powassan (N = 8)		Eastern equine encephalitis (N = 8)	
Characteristic	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Age group (yrs)†												
<18	65	(3)	72	(90)	4	(36)	0	(0)	0	(0)	0	(0)
18–59	1,165	(53)	4	(5)	1	(9)	6	(60)	3	(38)	4	(50)
≥60	974	(44)	4	(5)	6	(55)	4	(40)	5	(62)	4	(50)
Sex												
Male	1,403	(64)	38	(48)	5	(45)	4	(40)	6	(75)	4	(50)
Female	802	(36)	42	(53)	6	(55)	6	(60)	2	(25)	4	(50)
Period of illness onset												
January–March	3	(<1)	1	(1)	0	(0)	1	(10)	0	(0)	0	(0)
April–June	58	(3)	1	(1)	3	(27)	1	(10)	3	(38)	0	(0)
July–September	1,985	(90)	73	(91)	8	(73)	6	(60)	5	(62)	8	(100)
October–December	159	(7)	5	(6)	0	(0)	2	(20)	0	(0)	0	(0)
Clinical syndrome												
Nonneuroinvasive	858	(39)	4	(5)	5	(45)	4	(40)	1	(13)	0	(0)
Neuroinvasive	1,347	(61)	76	(95)	6	(55)	6	(60)	7	(88)	8	(100)
Encephalitis	620	(28)	63	(79)	3	(27)	4	(40)	5	(62)	6	(75)
Meningitis	565	(26)	12	(15)	2	(18)	1	(10)	2	(25)	1	(13)
Acute flaccid paralysis§	132	(6)	0	(0)	1	(9)	1	(10)	0	(0)	1	(13)
Other neurologic	30	(1)	1	(1)	0	(0)	0	(0)	0	(0)	0	(0)
Outcome												
Hospitalization	1,589	(72)	79	(99)	7	(64)	10	(100)	8	(100)	8	(100)
Death	97	(4)	3	(4)	0	(0)	0	(0)	0	(0)	2	(25)

Arboviruses

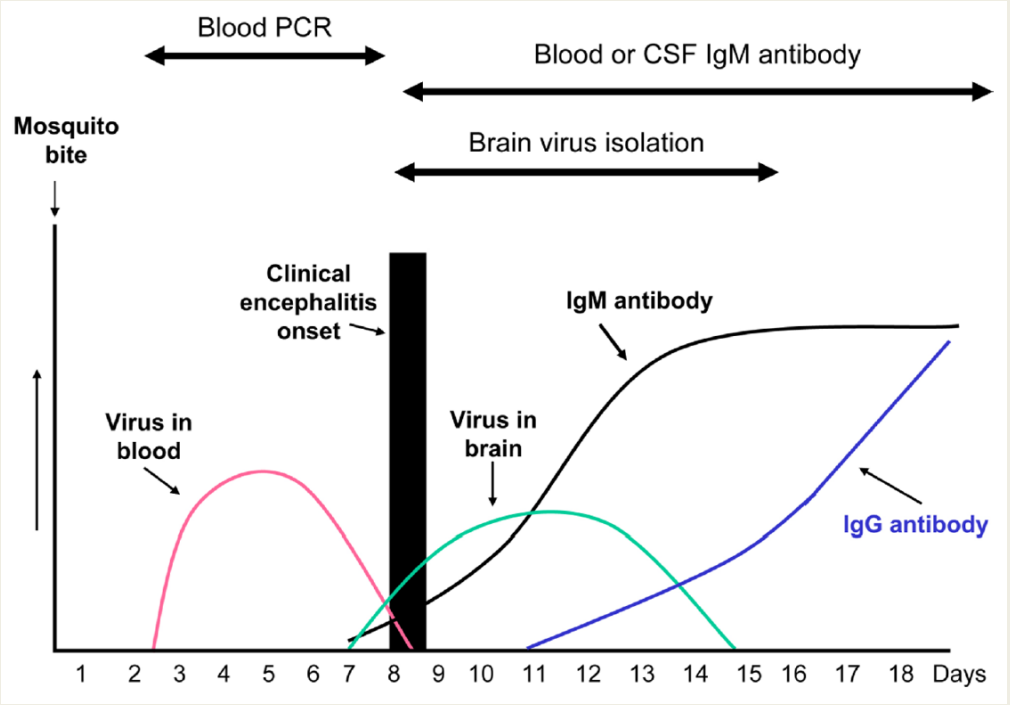
Diagnostics

- NAAT
  - *Blood → Viral replication/viremia precedes CNS involvement by 5-8 d*
    - Non-specific fever/rash
    - Not detectable by time of CNS symptom onset
  - *CSF → May be detected early in CNS symptoms*
    - Still poor sensitivity ~ 60-70%

NAAT not recommended as primary test for arboviral meningitis

…but…

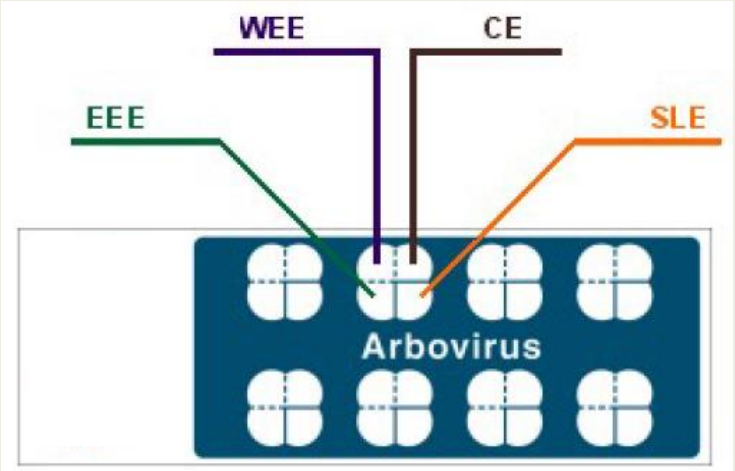
Specificity of NAAT is useful in epidemiologic studies

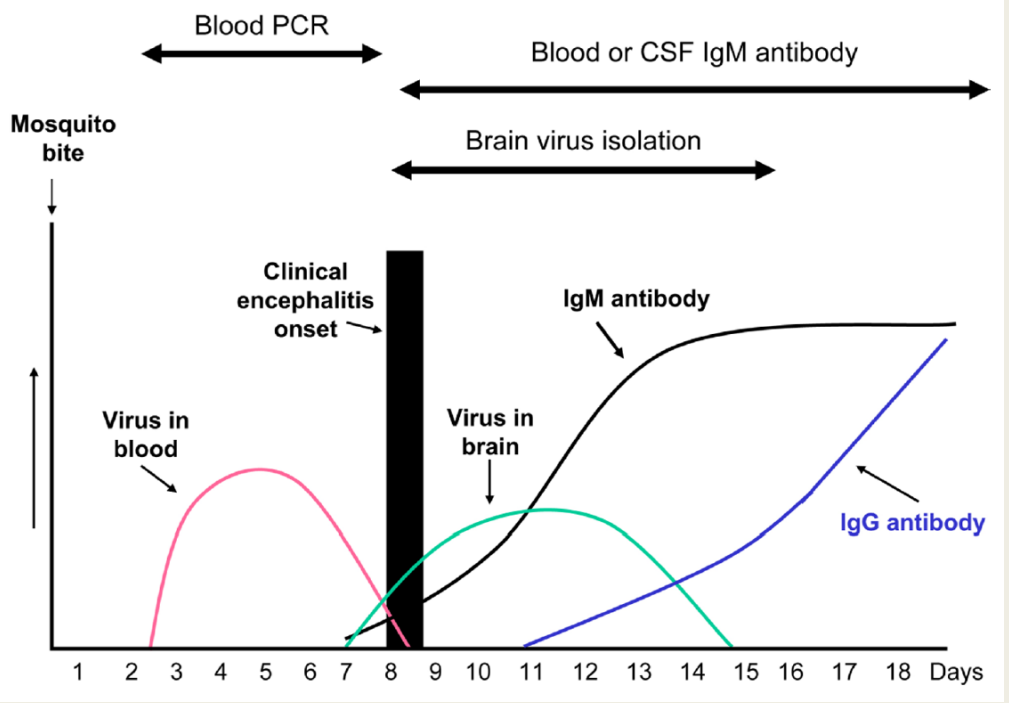


Arboviruses

Diagnostics

- Serology
  - *Blood → not specific for CNS involvement*
    - IgG persists for many years, if not life
    - IgM persists for 3-12 months
  - *CSF → Preferred method*
    - Intrathecal IgM indicates recent viral infection – likely cause
- Method - IFA
  - *Infected host cells spotted to slide*
    - Serum added, observe for fluorescence
    - Often multiple arbos on panel → same symptoms
  - *Cross-reactive!*
    - PRNT to definitively ID the specific arbovirus present





Herpesviruses

- Virus
  - *HSV, VZV, CMV, HHV-6, EBV*
- Epidemiology
  - *Meningitis resulting form re-activation of latent infection*
    - Competent – HSV, VZV
    - Compromised – VZV, CMV, HHV-6, EBV
- Symptoms
  - *Clinical presentation consistent with meningitis*
    - Fever, headache, photophobia
  - *Severe/life-threatening*
    - Immunosuppressed
    - Neonates

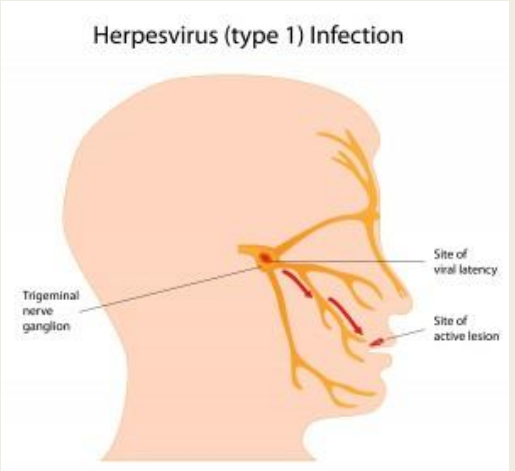
Herpesviruses

HSV

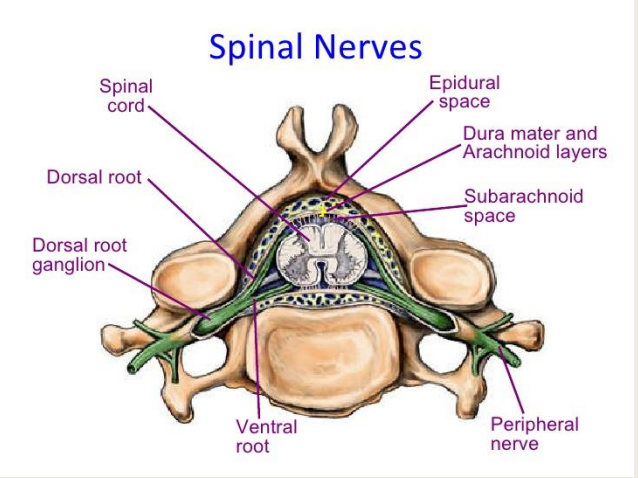
- Clinical
  - *HSV- 2 → Recurrent aseptic meningitis (Mollaret's)*
    - Immune-competent, young adults
    - Self-resolving, optimal treatment not established
  - *HSV- 1 → Sporadic encephalitis*
    - More common in compromised/HIV
    - Life-threatening, requires immediate treatment
      - *20% mortality, >95% of cases suffer long term neurologic defects*

Site of latency

HSV-1



HSV-2



Simplexa HSV (2014)

- Simplexa (Focus)
  - *Qualitative detection of HSV-1 and HSV-2 → 50 uL CSF, 1 h TAT*

Simplexa HSV (2014)

Simplexa HSV-1/2 Direct	Roche ASR HSV-1/2		
	Positive	Negative	HSV Type Indeterminate[c]
HSV-1			
Positive	11	0	1
Negative	0	85	3[d]
HSV-2			
Positive	37	1[d]	1
Negative	0	58	3[d]

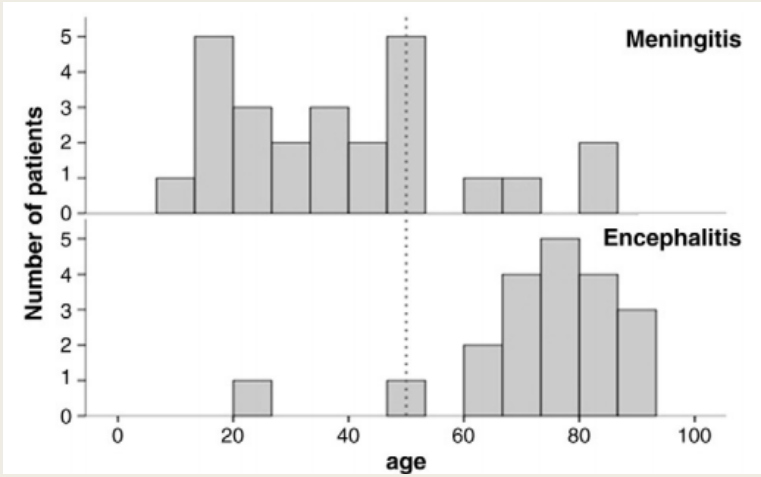
- **Performance**
  - *Single center*
    - 100 retrospective characterized CSF
    - Compared to LDTs
  - *Sensitivity: HSV-1: 100%, HSV-2: 100%*
  - *Specificity: HSV-1: 100%, HSV-2: 98.3%*
    - 3 samples resulted as IND by Roche were negative by 3rd molecular comparator (Artus HSV)

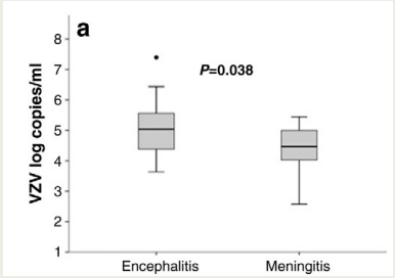
- **Clinical impact**
  - *Competent adults*
    - Recurrent HSV-2 meningitis common, self-limited no specific therapy
  - *Compromised adults*
    - HSV-1 severe encephalitis, require immediate treatment
  - *Children*
    - Important cause of neonatal meningitis → Assay off-label for blood, superficial (SEM screen)

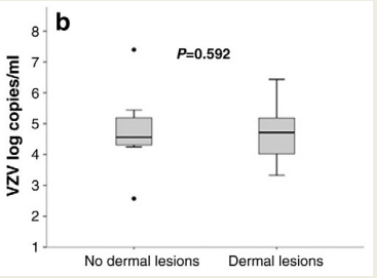
Herpesviruses

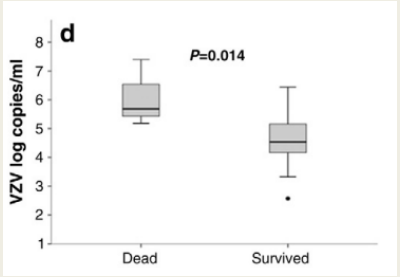
VZV

- Clinical
  - *Immunosuppressed → reactivation*
    - cerebellar ataxia, meningitis or encephalitis
    - Rash is present in only 42% of patients with confirmed VZV CNS disease
- Serology
  - *CSF IgM*
    - may be positive during asymptomatic reactivation or viremia episodes
    - Compare serum to CSF titer (?)
- NAAT
  - *Fast → <24 h*
  - *Sensitive → 10^2-10^3 copies/mL*
  - *Quant vs. Qual*
    - Qualitative typically associated with causality
    - Quant prognostic?









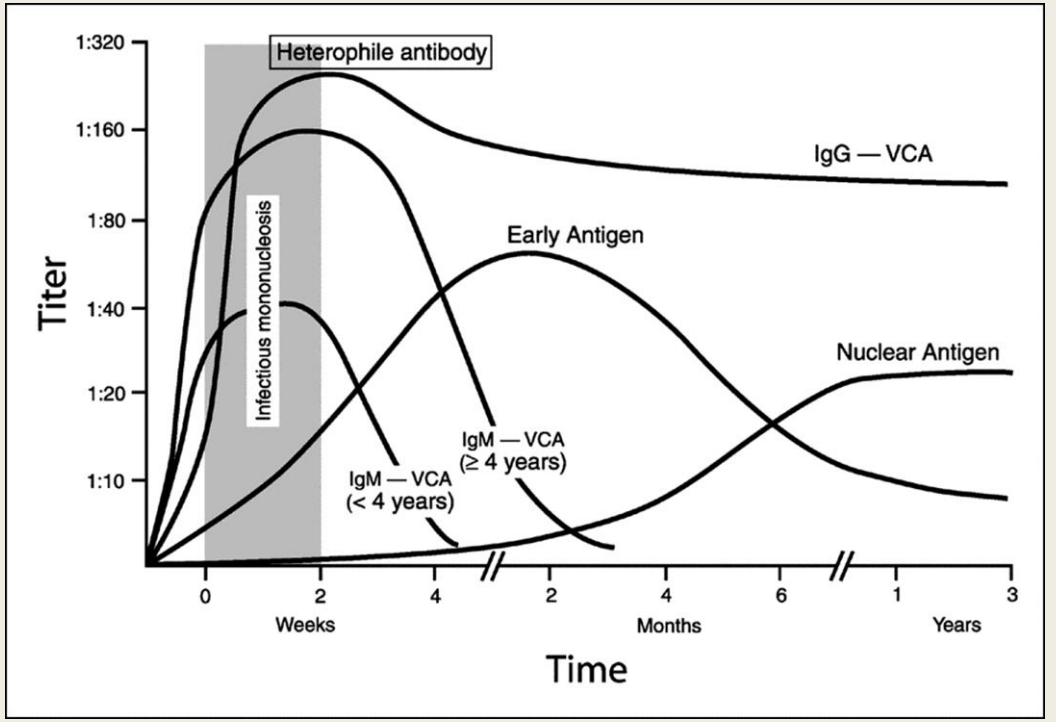
Diagnostic Microbiology and Infectious Disease 79 (2014) 174–177

Herpesviruses

EBV

- Virus
  - *~90% seropositivity*
  - *Establishes latency in B-cells*
    - Intermittent asymptomatic shedding in saliva

- Clinical
  - *Immunocompetent*
    - Acute IM → adenopathy, malaise
    - CNS symptoms follow primary infection (pediatric/young adult)
    - Aseptic meningitis, encephalitis
  - *Immunosuppressed → reactivation*
    - 20-100% Burkitt, 40% Hodgkin, 10% DBC lymphomas
    - >95% of primary central nervous system lymphomas (PCNSLs)



Serologic diagnosis: Heterophile Ab followed by VCA and EBNA

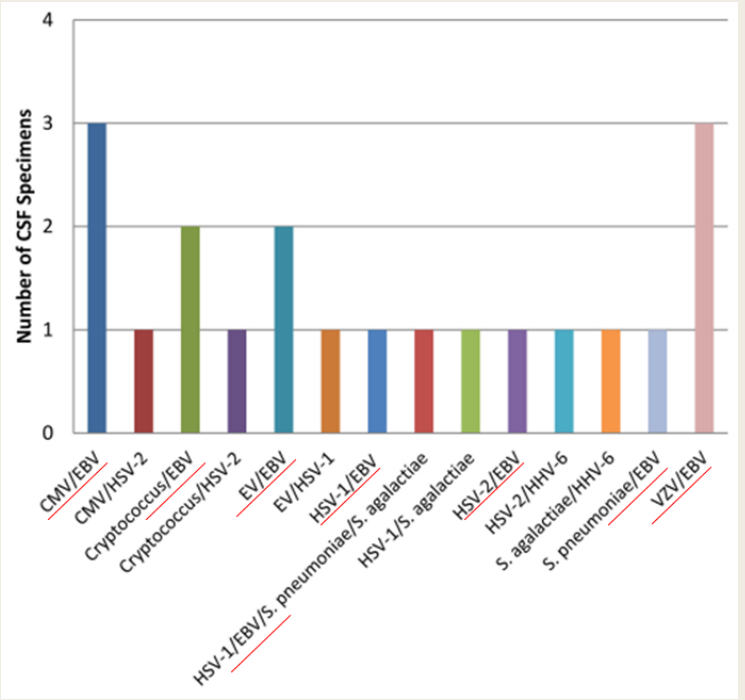
Herpesviruses

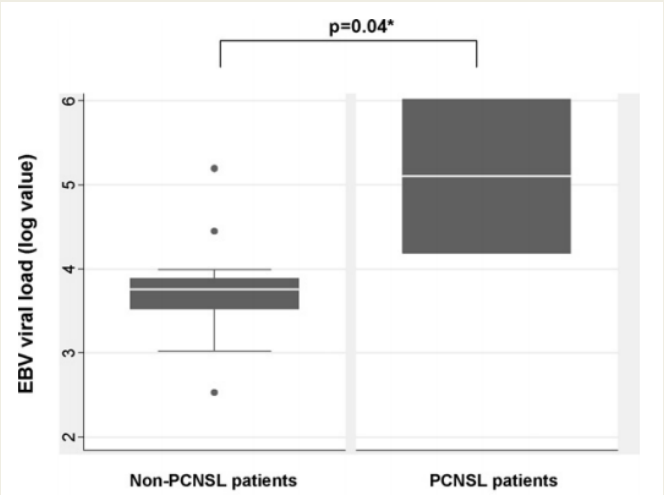
EBV

- Qualitative NAAT
  - *75-100% sensitive*
    - HIV-pos patients, histologically confirmed CNS lymphoma
  - *66-88% specific*
    - Positive result NOT correlated with increased risk of PCNSLs
    - 25-70% of EBV-positive specimens also pos for another likely pathogen
  - *30-50% PPV*

- Quantitative NAAT
  - *Can a threshold increase specificity?*
    - Threshold of 10^4 copies/mL → 96% specific
    - Not-standardized……lab-lab variability
  - *Comparison of serum vs. CSF VL?*

J. Clin. Microbiol. March 2016 vol. 54 no. 3 785-787



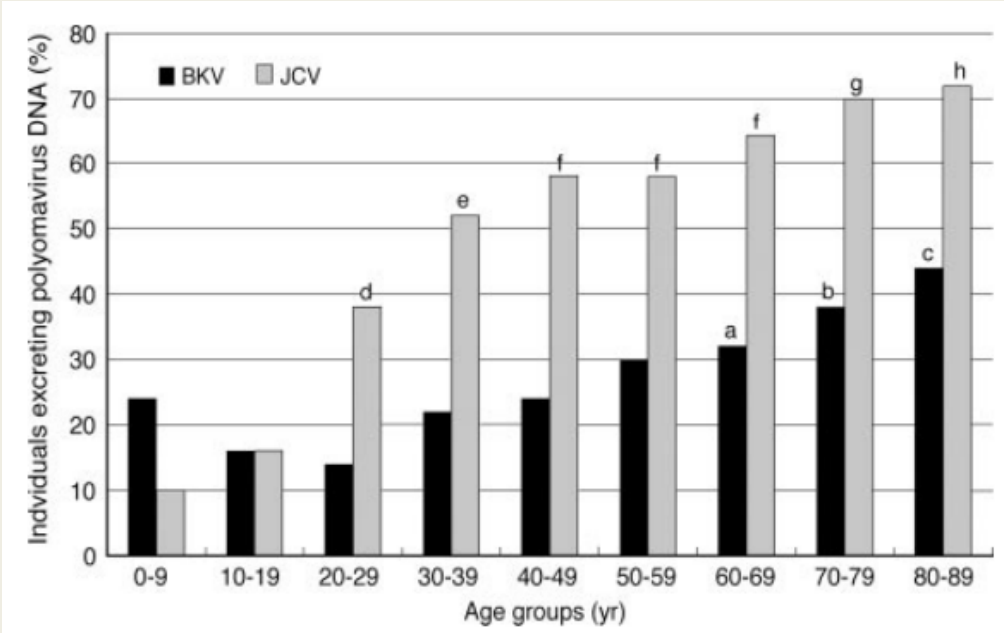


NAAT not extremely helpful, should not be used as sole means of diagnosis for CNS infections

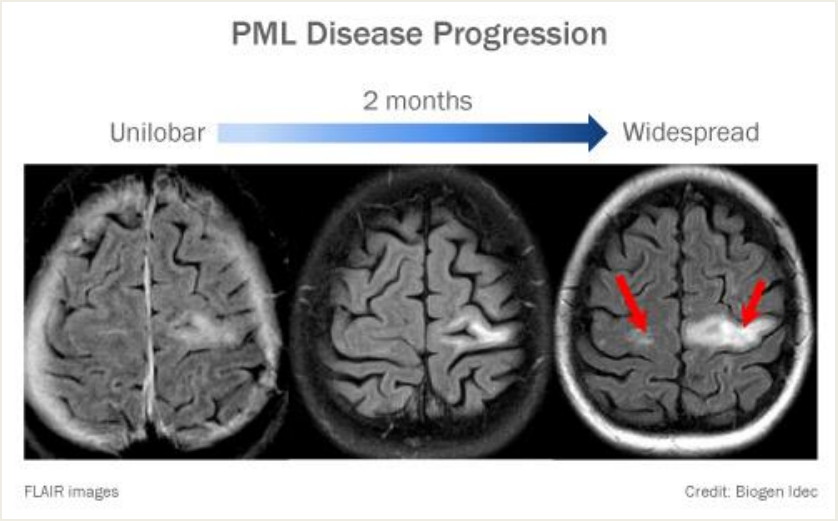
Polyomaviruses

- Virus
  - *JC, BK, circa 1970; nine others circa 2000-2005*
- Epidemiology
  - *Seroprevalence 60-95%*
    - JC/BK commonly shed in urine - asymptomatic
    - Compromised – severe focal organ disease
- Illness (JC)
  - *Progressive multifocal leukoencephalopathy (PML)*
    - Destructive viral replication
      - *demyelination of white matter in the brain*
      - *confusion, ataxia, paresis, and death if untreated*
    - Immunosuppressed
      - *AIDS, HSCT, therapy for MS (natalizumab)*

% of healthy patients with detectable BK/JC in Urine



JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2007, p. 193–198



Polyomaviruses

JC

- Culture
  - *Non-cultivable in routinely used cell lines*
- Serology
  - *Not useful for diagnosis, 60-90% seropositivity*
  - *Potential to screen/stratify risk for those considering certain MS therapies*
- NAAT
  - *Fast → <24 h*
  - *Sensitivity is key!!*
    - 10^1-10^2 copies/mL (95% sensitive)
    - 10^2-10 copies/mL (75% sensitive)
  - *Surrogate less invasive specimens…CSF vs. Blood, Urine?*
    - Urine → detected in 40-70%, not correlated with PML
    - Blood → detected in 0.3-1%, none developed PML
  - *No FDA-cleared assays, reference labs LDTs*

Diagnosic criteria for PML

Definite (etiological) diagnosis:

CSF-confirmed PML:

a. Clinical and MRI findings consistent with PML and

b. Evidence of JCV DNA in CSF

Tissue-confirmed PML:

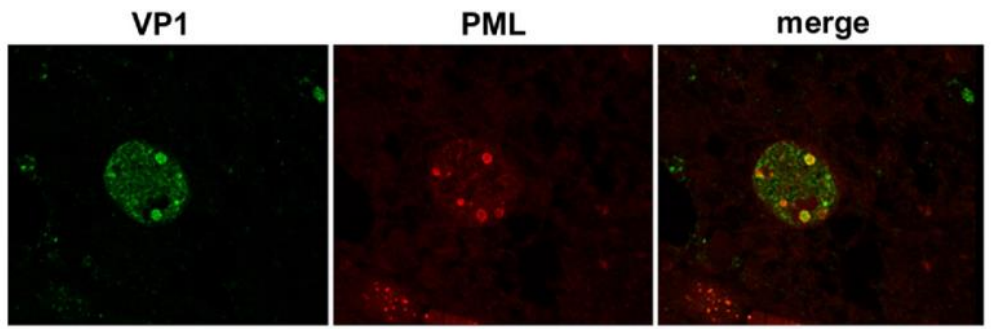
a. Evidence of PML neuropathology in brain tissues (biopsy or autopsy) with JCV DNA or protein detected by in situ techniques.

Presumptive (clinical) diagnosis:

a. Evidence of typical clinical and MRI findings and

b. Brain biopsy and lumbar puncture either not performed or JCV DNA not detected in CSF.

Lancet Infect Dis. 2009 October ; 9(10): 625–636



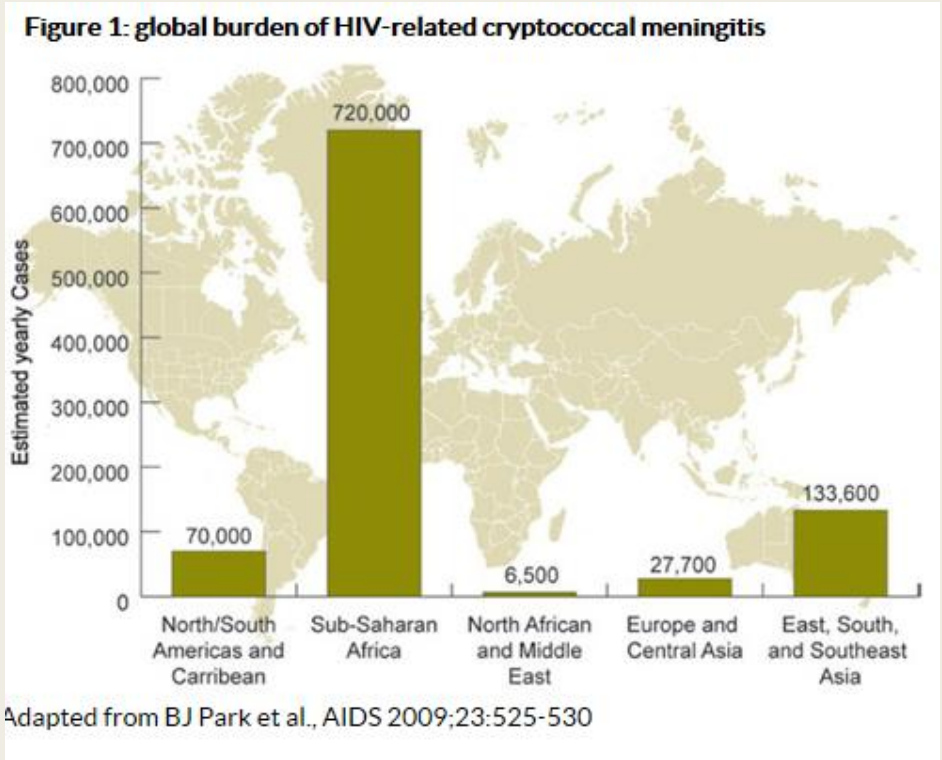
The American Journal of Pathology, Vol. 180, No. 3, March 2012

FUNGAL MENINGITIS

Clinical impact of viral meningitis

- **Epidemiology**
  - *Historically rare compared to viral/bacterial*
    - Increasing prevalence with increasing immunosuppressed population
      - *HIV/AIDS, hematologic malignancies, direct spinal surgeries/therapies*
    - Pathogens
      - *Cryptococcus - >90% of fungal CNS infections*
      - *Dimorphic fungi – Coccidioides, Blastomyces*
      - *Filamentous fungi – "dematiaceous molds"*

- **General risk factors**
  - *Age*
  - *Immunecompromise (HIV ~100-fold higher incidence of Crypto)*
  - *Exposure*
    - Geographic location
    - Medical procedures



Detection methods

- Culture
- Antigen
- Serology
- NAAT/PCR

Best method depends on…

specific fungus, available tests, specimen

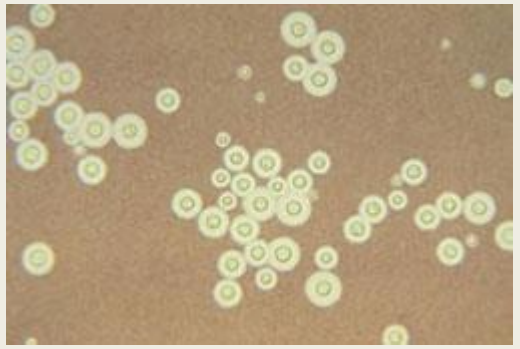
Detection methods

Culture

- Direct exam of specimen
  - *Low yield, not routinely performed*
- Plating
  - *> 2mL → centrifuge, plate entire pellet*
    - Supernatant can be used for serologic tests
  - *< 2mL → plate entire volume to fungal culture media*
- Sterile source
  - *Critical to differentiate contamination from true infection*
    - Do NOT streak inoculum → consider growth only at inoculation site

CSF culture for Fungi is typically low yield, augment with second approach when available

Detection methods



Cryptococcus

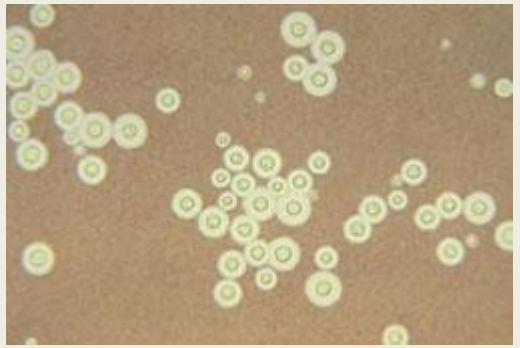
- Direct exam of specimen
  - *India ink smear → Poor sensitivity*
- Culture
  - *Highly dependent on specimen volume/abx exposure*
- Cryptococcal Ag
  - *Latex agglutination – capsular polysaccharide*
    - Most sensitive method for diagnosis of Cryptococcal meningitis
      - *CSF*
      - *Serum*
    - Persists after resolution of symptoms
      - *Positive in culture-negative/NAAT negative samples*
      - *Not a test of cure!*
    - Can cross-react with other capsulated yeast → *Tricochsporon, Rhodotorula*

Laboratory findings in CSF before and after antifungal therapy[a]

Time of findings	Leukocyte (10^6/liter)	No. of positive fungal cultures (%)	Cryptococcal titer
Before therapy	132.4 ± 228.5	20 (69.0)	1,049.1 ± 4.1
After therapy	12.9 ± 11.2	0 (0.0)	238.4 ± 4.4

JOURNAL OF CLINICAL MICROBIOLOGY, June 2005, p. 2989–2990

Detection methods



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JOURNAL OF CLINICAL MICROBIOLOGY, June 2005, p. 2989–2990

Efficiency of different techniques in the diagnosis of cryptococcal meningitis in different hosts[a]

No. of patients	Host condition	No. positive by CSF Ag (%)	No. positive by CSF culture (%)	No. positive by India ink (%)	No. positive by serum Ag (%)
401	AIDS	307/333 (92.2)	380/401 (94.7)	302/375 (80.5)	181/183 (98.9)
331	HIV negative, immunocompromised	214/219 (97.7)	251/282 (89)	139/231 (60.2)	129/148 (87.2)
116	Immunocompetent	72/78 (92.3)	91/102 (89.2)	89/116 (76.7)	181/183 (98.9)
848	Overall total	593/630 (94.1)	722/785 (92)	430/722 (59.6)	310/331 (93.6)

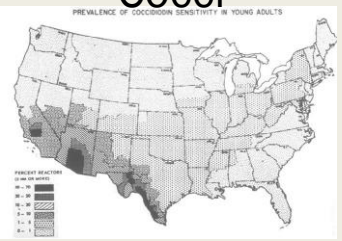
JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2005, p. 5828–5829

Detection methods

Blasto

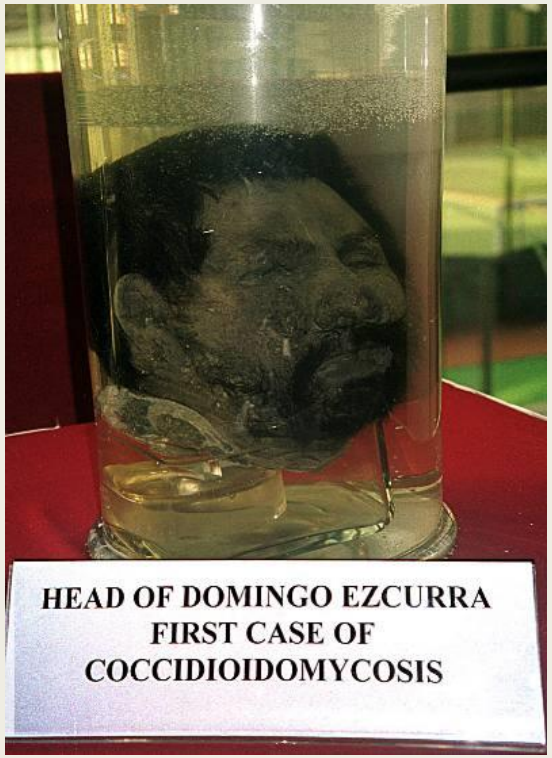
Histo

Cocci



Dimorphs

- Coccidioides, Blastomyces, Histoplasma
  - *CNS infection secondary to resp. infection*
  - *More common in compromised host*
    - Histo – 5-10% of disseminated infections
    - Blasto – <5% of disseminated infections
    - Coccidioides – **30-50%** of disseminated infections
- Culture - Poor sensitivity from CSF, long TAT, ***HANDLE WITH CARE!***
- Serology – Poor sensitivity in compromised host, high seroprevalence
- Antigen tests
  - *Blasto/Histo*
    - Urine antigen test >90% sensitive for disseminated disease
    - Cross-reactive (Blasto, Histo, Paracoccidioides)

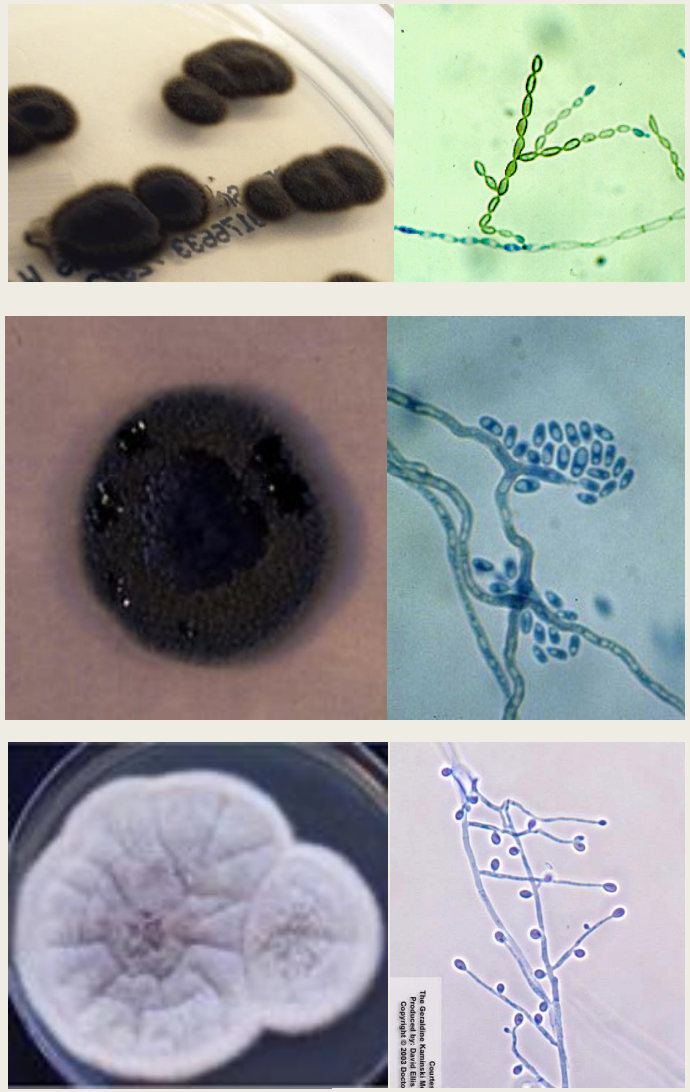


Detection methods

Dematiacious

- "Dark walled" fungi, contain melanin
  - *Saprophytic → soil/decaying plant material*
  - *Filamentous and yeast-like organisms*
    - Cladophilophora bantiana
      - *Inhalation → neroinvasive/parenchymal growth*
    - Exophiala → yeastlike
      - *Traumatic introduction → injury, lines, ports*
    - Scedosporium
      - *Inhalation → neurotropic*

- Opportunistic skin/soft tissue and respiratory pathogens
  - ***Tropism for CNS***
  - ***Affect young/healthy individuals***



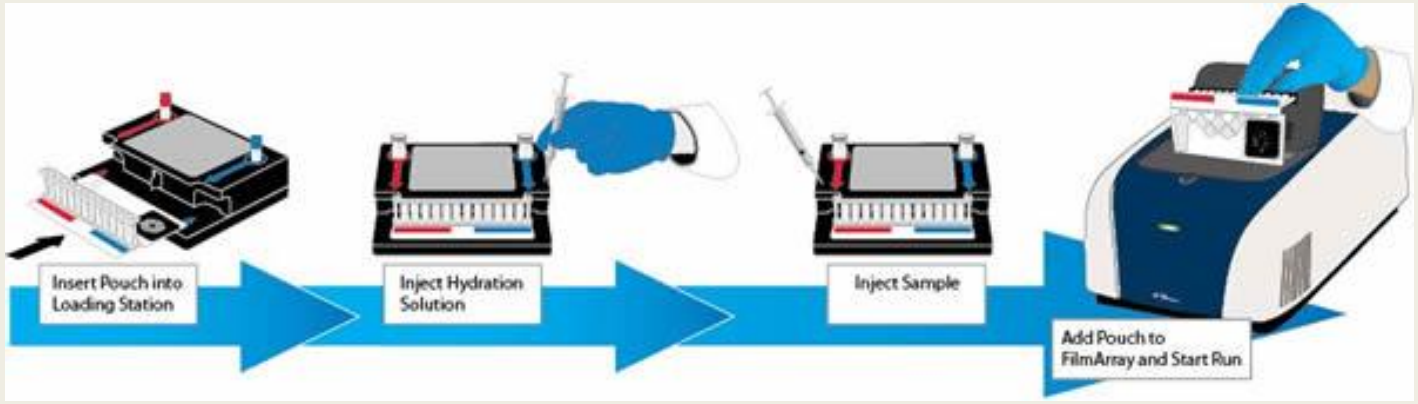
MOLECULAR DIAGNOSTICS

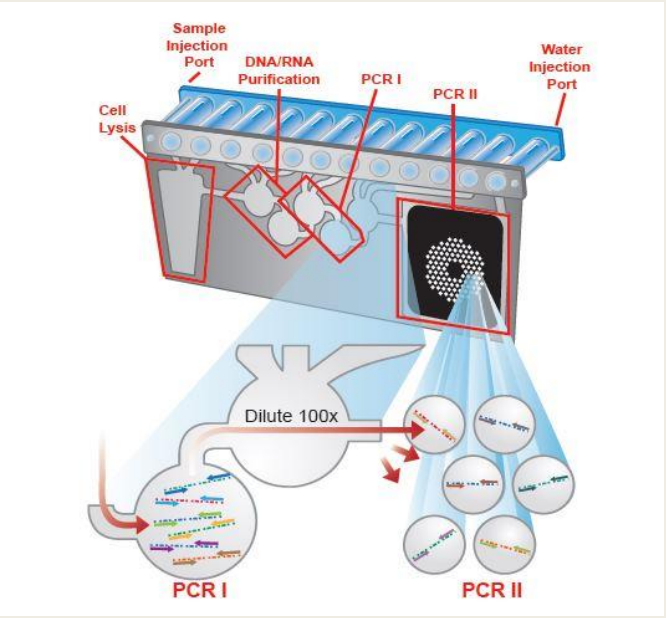
Syndromic panels

“Syndromic panel”

FilmArray ME (BioFire, 2015)

- Simultaneous detection of 14 targets → 200 uL CSF, 1 h TAT
  - *Bacteria → E. coli K1, H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, S. pneumoniae*
  - *Viruses → CMV, VZV, HSV-1, HSV-2, HHV-6, Enterovirus, Human Paraechovirus*
  - *Fungi → Cryptococcus neoformans/gattii*





FilmArray ME (2015)

- **Performance**
  - *Single center*
    - 174 retrospective characterized CSF
    - Compared to LDTs (viral), culture (bacterial)
      - *Discordant results tested with third LDT NAAT*

TABLE 1 Distribution of organisms identified by conventional methods and the FilmArray meningitis/encephalitis (FA ME) panel

Organism identification[a]	Conventional detection, no.	FA ME panel detection, no.	Baseline agreement, no.	Resolution result, no.[b]			Sensitivity, % (95% CI)[c]	Specificity, % (95% CI)[c]
				FA+/R+	FA+/R−	FA−/R+		
Bacteria								
H. influenza	4	5	4	1	0	0	100 (47.8–100)	100 (97.4–100)
S. pneumoniae	3	6	3	2	1	0	100 (47.8–100)	99.3 (96.1–100)
S. agalactiae	1	5	1	2	2	1	66.7 (9.4–99.2)	98.6 (95.0–99.8)
Escherichia coli	1	1	1	NA[d]	NA	NA	100 (2.5–100)	100 (97.5–100)
Listeria monocytogenes	0	0	1	NA	NA	NA	NA	100 (97.5–100)
Neisseria meningitides	1	1	1	NA	NA	NA	100 (2.5–100)	100 (97.5–100)
Bacteria not in the FA ME panel[e]	7	0	7	NA	NA	NA	NA	NA

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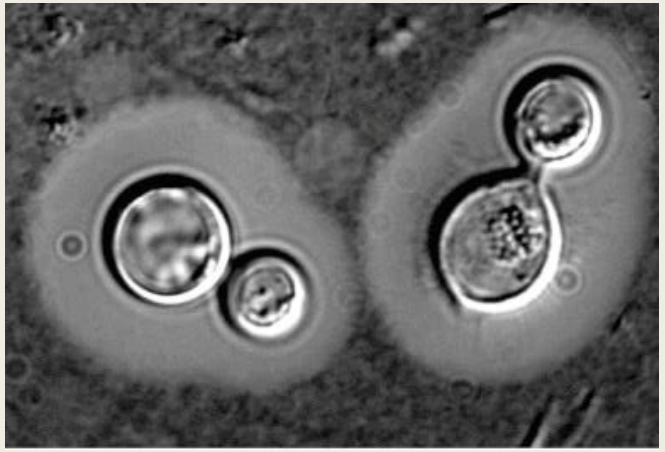
Viruses								
EV	37	37	36	1	0	1	97.4 (86.2–99.9)	100 (69.2–100)
HSV-1	12	13	11	0	2	1	92.9 (66.1–99.8)	98.0 (89.1–99.9)
HSV-2	29	29	29	NA	NA	NA	100 (88.1–100)	100 (82.4–100)
HHV-6	13	18	12	6	0	1	94.7 (74.0–99.9)	100 (92.6–100)
VZV	32	32	32	NA	NA	NA	100 (89.1–100)	100 (79.4–100)
CMV	7	4	4	0	0	3	57.1 (18.4–90.1)	100 (91.4–100)
EBV	13	25	11	5	9	1	94.1 (71.3–99.9)	84.2 (72.1–92.5)
PV	0	1	0	1	0	0	100 (2.5–100)	100 (92.5–100)
Yeast								
C. neoformans/gattii	14	9	8	1	0	0[f]	64.3 (35.1–87.2)	NA
Total	174	186	161	19	14	8	92.8 (88.2–96.0)	92.8 (88.2–96.0)

FilmArray ME (2015)



- **Potential pitfalls**
  - *Cryptococcus*
    - 64% sensitive compared with CrAG
      - *All discordants were negative by alternative NAAT and culture*
      - *CrAG more sensitive than NAAT? AG persist?*

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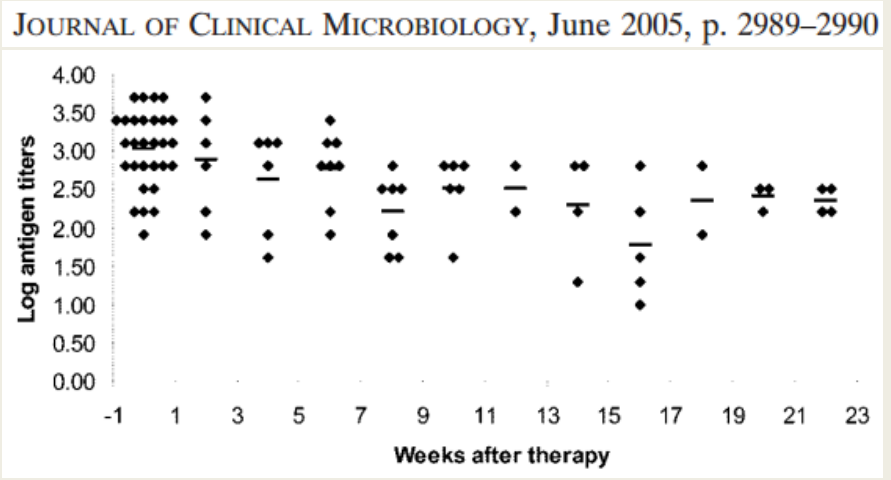

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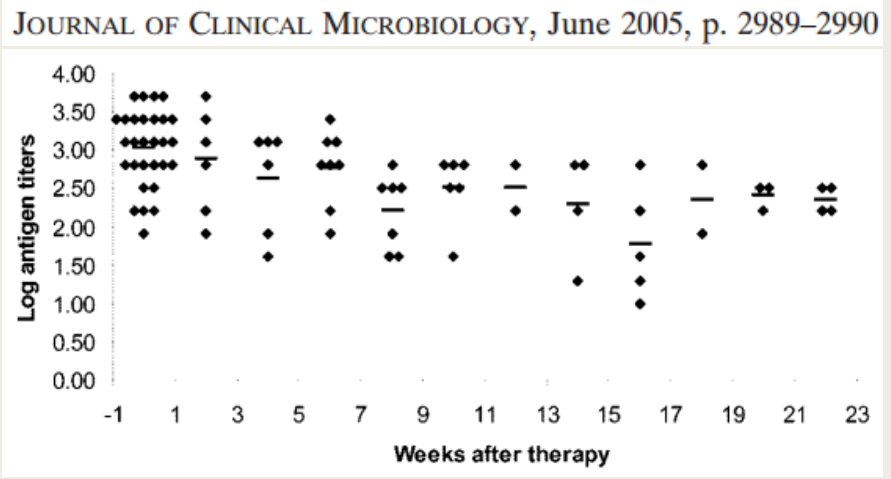
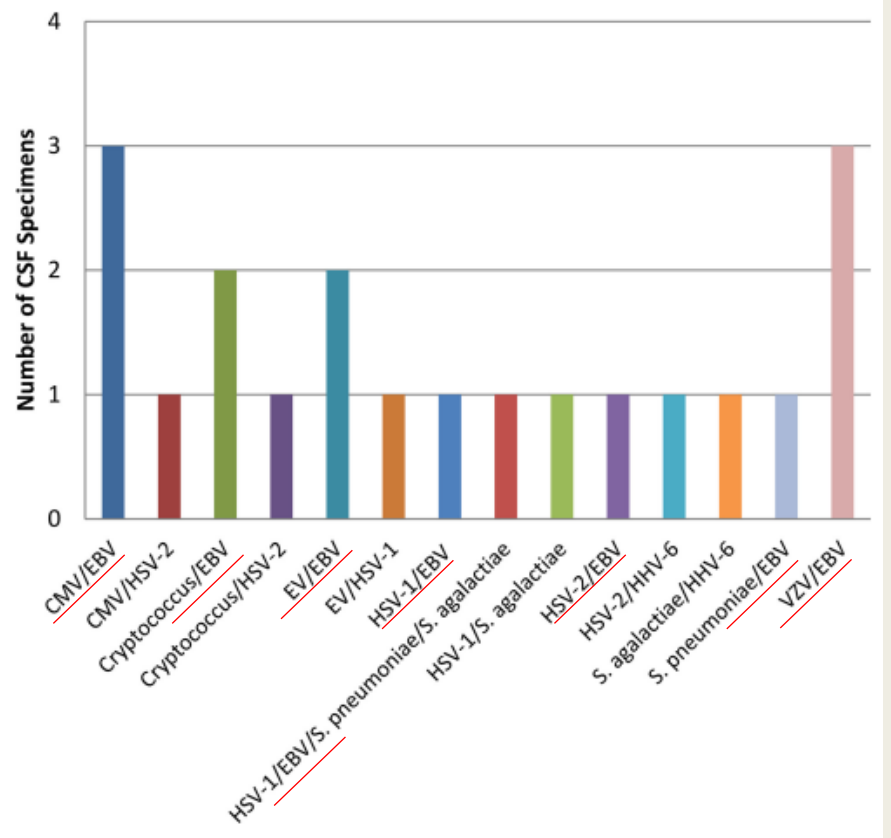
      - *Lymphocytic infiltrates → latent virus?*
      - *CMV? HHV-6? → clinical significance?*





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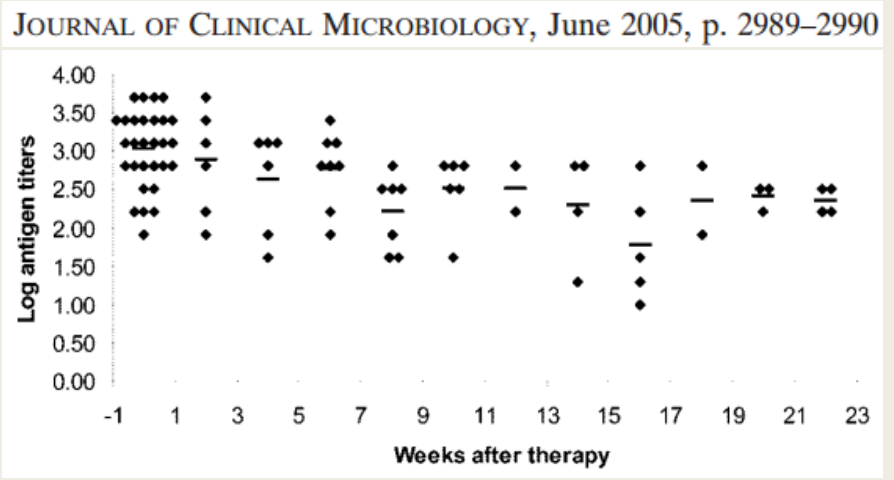
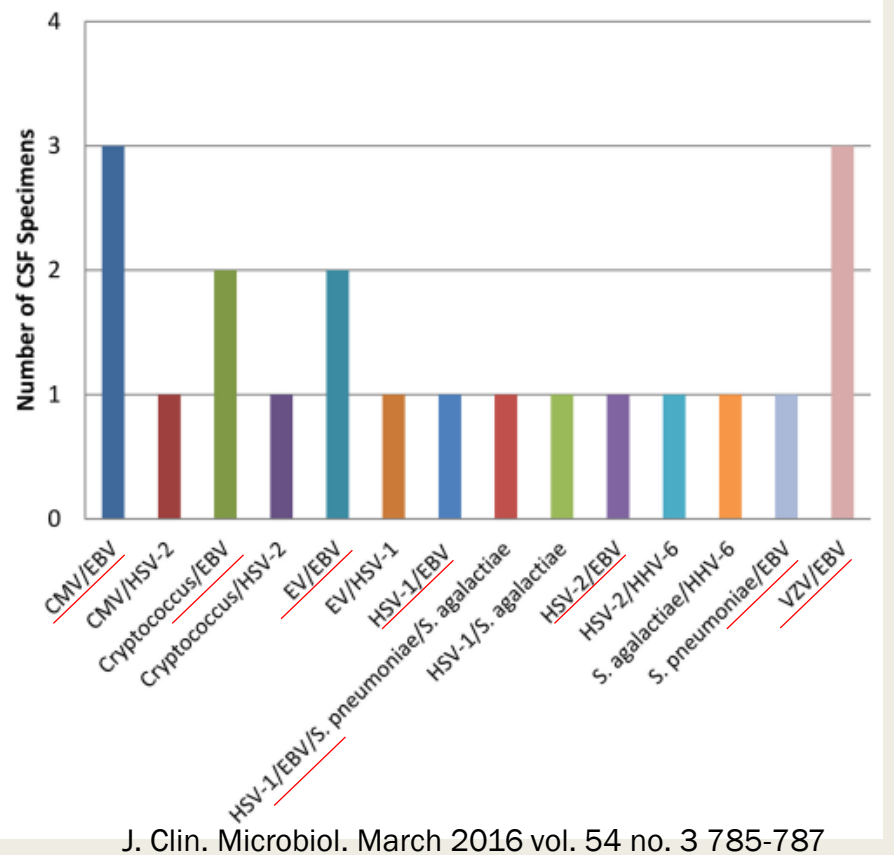
  - *S. pneumoniae*
    - 99% (1544/1556) specific but….
      - *Only 5/12 FP confirmed by alternative NAAT*
      - *9 TP, 3 FP → PPV 75%*
        - Data from package insert

With low prevalence population, specificity is key!!!!!!!





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FilmArray ME (2015)

- **Utilization**
  - *Pediatrics*

    - Rapid, effective method to determine cause of symptoms
      - *Enterovirus/HPeV vs. HSV vs. bacterial*
  - *Adult outpatient, acute onset*

    - Rapid method for HSV, but more $$$ than Simplexa HSV
      - *Consider clinical picture (severe symptoms, elderly), do results fit?*
      - *S. pneumoniae? Other herpesviruses?*
  - *Compromised patient*

    - Rapid, but is it comprehensive?
      - *14/110 (13%) positive bacterial cultures were on-panel targets*
    - Analytical vs. clinical specificity for herpesviruses
  - *Inpatient with hardware*

    - No! Common bugs not on panel (CoNS, *P. aeruginosa, Enterococcus, Acinetobacter*)

ID	# cultures	% cultures
S. epidermidis	39	35.5%
CoNS	5	4.5%
Corynebacterium	1	0.9%
P. acnes	4	3.6%
Bacillus spp.	2	1.8%
Micrococcus	1	0.9%
S. pneumoniae	4	3.6%
*N. meningitidis**	3	2.7%
E. coli	4	3.6%
S. marcessens	3	2.7%
Enterococcus	6	5.5%
P. aeruginosa	7	6.4%
Enterobacter spp.	4	3.6%
S. agalactiae	2	1.8%
S. pyogenes	1	0.9%
Acinetobacter	3	2.7%
Viridans gr. Strep.	5	4.5%
S. aureus	4	3.6%
P. mirabilis	1	0.9%
Mixed pathogens	5	4.5%
Candida spp.	2	1.8%
C. neoformans	4	3.6%
Total	110	

Conclusion

- **Meningitis remains a common, potentially serious condition**
  - *Critical to get result to clinician as fast as possible*
    - Major impact on care and management (antibiotics, antivirals? supportive care?)
  - *No single approach is sufficient to detect all causes*
  - *In choosing orderable test consider*
    - Symptoms
    - Patient population
    - Current and previous infections/anatomic sites
    - Geographic locale
  - *Molecular tests are typically the most sensitive method for diagnosis however…*
    - Few FDA-cleared options
    - “Only find what you are looking for” – potential for false sense of security
    - Must always be accompanied by culture

THE END

Are we still awake?