# BATTLING INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Organisms, Risk Factors, and Laboratory Diagnostics

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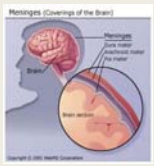
## Outline

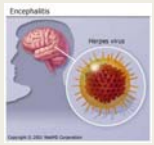
- Overview of meningitis
- Address P.A.C.E. Goals
  - *Identify organisms commonly associated with CNS infection*
  - *Discuss the factors which put patients at risk for these infections*
  - *Explain the strengths and weaknesses of current diagnostic methods*
- Clinical and financial impact of rapid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

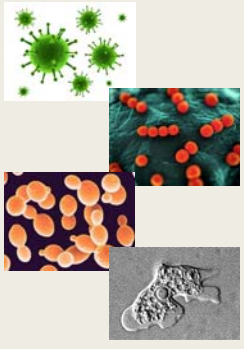
**Routes of infection**

- 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*

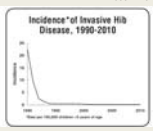
| Demographic | Common Bacterial Etiology |
|---|---|
| Neonate | *S. agalactiae, E. coli, L. monocytogenes* |
| Infants, young children | *H. influenzae, S. pneumoniae, N. meningitidis* |
| Young adult | *N. meningitidis* |
| Adult | *S. pneumoniae, N. meningitidis* |
| Elderly | *S. pneumoniae, L.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

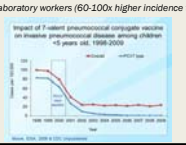
## Epidemiology

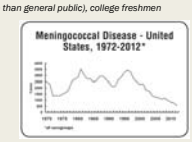
- **Initial surveys in early 1980's**
  - *Attack rate of 3.0-6.0 cases/100,000*
    - 10-20x higher for children <1 yoa
  - *Common agents…source of infection?*
    - 75-85% → *H. influenzae, S. pneumoniae, N. meningitidis*
    - 2-5% → *S. agalactiae (neonates, now elderly as well) L. monocytogenes*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

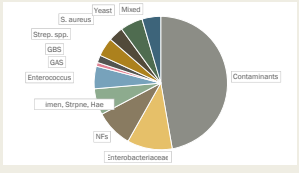





## Epidemiology

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

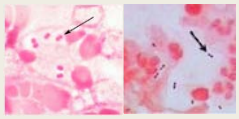



| ID | # cultures | % cultures |
|---|---|---|
| *S. epidermidis* | 39 | 35.5% |
| *CoNS* | 5 | 4.5% |
| *Corynebacterium* | 1 | 0.9% |
| *P. acnes* | 4 | 3.6% |
| *Bacillus spp.* | 2 | 1.8% |
| *Micrococcus* | 1 | 0.9% |
| *S. pneumoniae* | 4 | 3.6% |
| *N. meningitidis** | 3 | 2.7% |
| *E. coli* | 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**

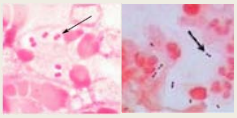
## Detection methods



**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**

- Critical value!
  - *Critical value → establish acceptable TAT for reporting (<2 h)*
    - Cellularity (RBCs, PMN vs. Monos)
    - 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

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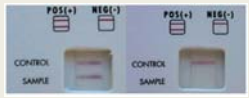


**Antigen**

- 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?*

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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[a] | 4 | 1 (25) | 0 (0) |
| Total | 42 | 13 (31) | 5 (12) |

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

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      - *"No substantial benefit beyond concentrated Gram stain"*
- MIC.22550 – Back-up cultures required on <u>both</u> AG-positive and negative CSF specimens

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- "Gold standard"
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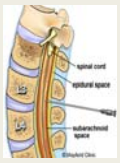
    - Aerobic culture (BAP, CHOC) + Thio broth
- Sensitivity?

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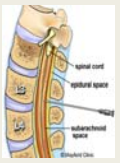
- Sensitivity?
  - *80-95%*
  - *Factors impacting culture sensitivity*
    - Organisim: 95% *H. flu*, 90% *S. pneumo*, 80% *N. mening*
    - CSF volume: some infections ≤$10^3$ CFU/mL
    - Abx usage: 60-80% *decrease* in sensitivity
    - Gold standard: Cytology (>1000 WBC/uL, >80% PMN)
- Blood culture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

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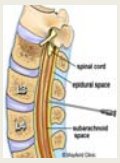


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Sensitivity of GS, BC, CSF Culture

| | |
|---|---|
| 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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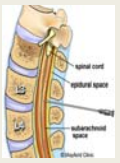


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**NAAT/PCR**

- 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*
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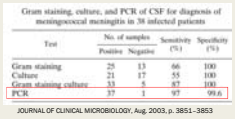

Gram staining, culture, and PCR of CSF for diagnosis of meningococcal meningitis in 38 infected patients

| Test | No. of samples Positive | Negative | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| 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

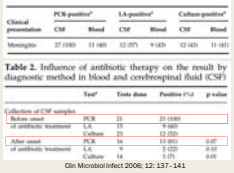

Table 2. Influence of antibiotic therapy on the result by diagnostic method in blood and cerebrospinal fluid (CSF)

Clin Microbiol Infect 2006; 12: 137–141

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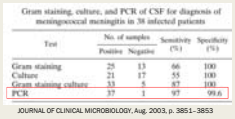
- PCR considered "gold standard" for *N. meningitidis* in UK
  - *Observed a 56% increase in lab-confirmed meningococcal disease*



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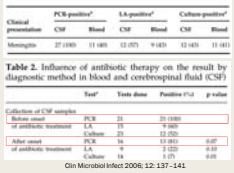

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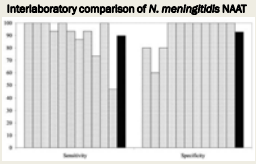
## Detection methods


**NAAT/PCR**

- Obstacles to NAAT
  - *Few FDA-cleared options → LDT?*
    - Complex to design
      - *Inhibitors - Elevated proteins, globulin, cellular infiltrates, hemin*
      - *Targets – 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**

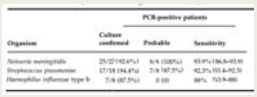


JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 144–149

## Detection methods


**NAAT/PCR**

- Obstacles to NAAT
  - *Few FDA-cleared options → LDT?*
    - Complex to design
      - *Inhibitors - Elevated proteins, globulin, cellular infiltrates, hemin*
      - *Targets – 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?*




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

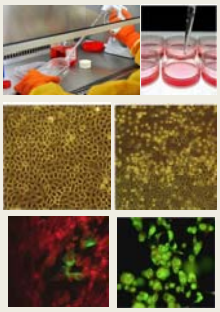
| Virus | Effectiveness[b] of diagnosis by: PCR | | Serology[c] | | Culture of specimens | | | |
|---|---|---|---|---|---|---|---|---|
| | Serum | CSF | Serum | CSF | Throat | Rectal | Blood | CSF |
| Arboviruses | +[d] | +[d] | ++ | ++ | – | – | ++ | +/– |
| WNV | +[d] | +[d] | ++ | ++ | – | – | – | – |
| 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**

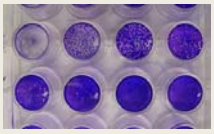
- Poorly sensitive
  - *CSF culture yield especially low, not typically recommended for diagnosis*
    - HSV ~ 20% sensitive, EVs 30-35% sensitive, JC not cultivable using standard cell lines
  - *Potentially due to presence of neutralizing Ab, low VL in CSF*
    - Preventing uptake of viruses by host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

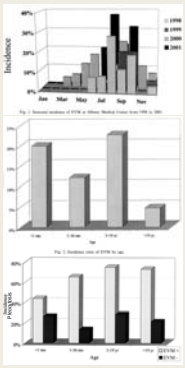
Increasing Ab concentration →



PRNT

## Enteroviruses

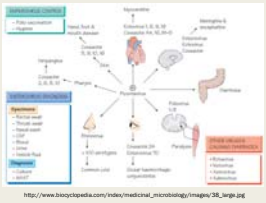
- Virus
  - *Picornaviridae (enterovirus, echovirus, coxsackievirus)*
    - Non-enveloped >60 serotypes
- Epidemiology
  - *Summer-fall, primarily in children <5 yoa*
    - Transmitted in feces, saliva, environmental sources (water)
  - *80-90% of aseptic meningitis when etiology is found*
- Symptoms
  - *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*



## 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/imagesv/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

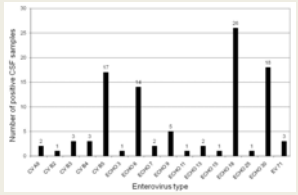

## Xpert EV (2009)

- **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. 526-533

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

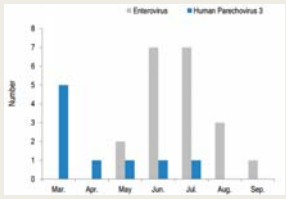
| Site | No. of samples | | | | | Sensitivity | Specificity |
|---|---|---|---|---|---|---|---|
| | Total | TP | FP | TN | FN | | |
| 1 | 15 | 2 | 0 | 13 | 0 | 100 | 100 |
| 2 | 34 | 8 | 0 | 24 | 2 | 80 | 100 |
| 3 | 44 | 3 | 0 | 41 | 0 | 100 | 100 |
| 4 | 84 | 27 | 0 | 57 | 0 | 100 | 100 |
| 5 | 22 | 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

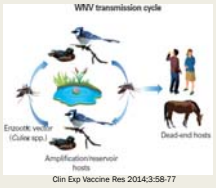
## Xpert EV (2009)

- **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 | | Difference | P |
|---|---|---|---|---|
| | ≤24 h (n = 17) | >24 h (n = 33) | | |
| 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

| Characteristic | West Nile (N = 2,205) No. | (%) | La Crosse (N = 80) No. | (%) | Jamestown Canyon (N = 11) No. | (%) | St. Louis encephalitis (N = 10) No. | (%) | Powassan (N = 8) No. | (%) | Eastern equine encephalitis (N = 8) 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

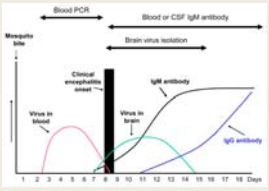
**Diagnostics**

- 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%

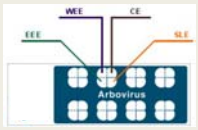
*NAAT not recommended as primary test for arboviral meningitis*

*...but...*

*Specificity of NAAT is useful in epidemiologic studies*

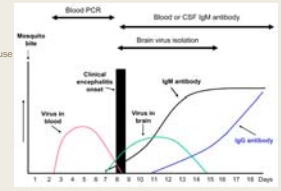



## Arboviruses




**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

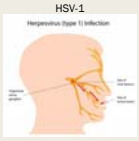
## Herpesviruses

**HSV**

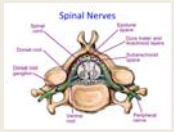
- Clinical
  - *HSV- 2 → Recurrent aseptic meningitis (Mollaret's)*
    - Immune-competent, young adults
    - Self-resolving, optimal treatment not established
  - *HSV- 1 → Sporadic encephalitis*
    - More common in compromised/HIV
    - Life-threatening, requires immediate treatment
      - *20%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

- **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)

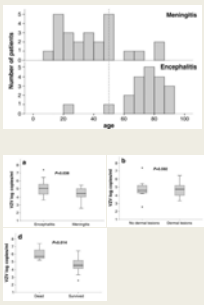
| Simplexa HSV-1/2 Direct | Roche ASR HSV-1/2 HSV Type Positive | Negative | Indeterminate[c] |
|---|---|---|---|
| HSV-1 | | | |
| Positive | 11 | 0 | 1 |
| Negative | 0 | 85 | 3[d] |
| HSV-2 | | | |
| Positive | 37 | 1[d] | 1 |
| Negative | 0 | 58 | 3[d] |

- **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?



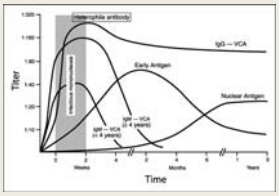



Diagnostic Microbiology and Infectious Disease 79 (2014) 174–177

## 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

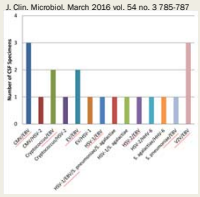
## Herpesviruses

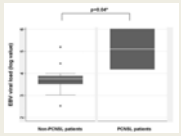
**EBV**

- 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?*

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

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

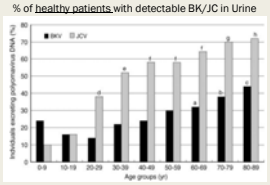


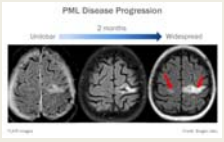

## Polyomaviruses

- 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

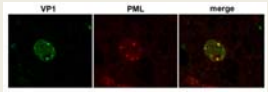



## Polyomaviruses

**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*


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

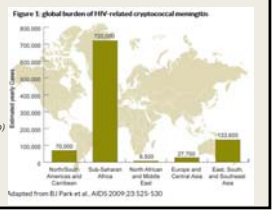



The American Journal of Pathology, Vol. 180, No. 3, March 2012

# 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



Adapted from BJ Park et al., AIDS 2009;23:525-530

## 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*

| 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

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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*

| 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

**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***

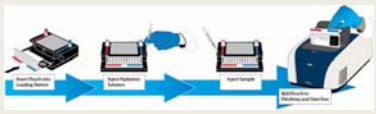
# MOLECULAR DIAGNOSTICS

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] FA+/R+ | FA+/R− | FA−/R+ | Sensitivity, % (95% CI)[c] | Specificity, % (95% CI)[c] |
|---|---|---|---|---|---|---|---|---|
| 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) | 99.5 (96.1–100) |
| *S. agalactiae* | 1 | 3 | 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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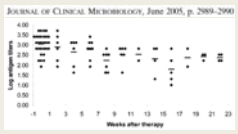


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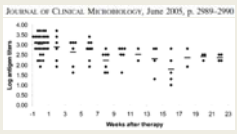
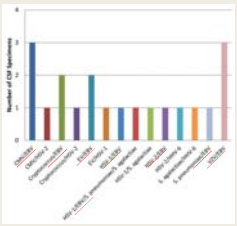
      - *Lymphocytic infiltrates → latent virus?*
      - *CMV? HHV-6? → clinical significance?*

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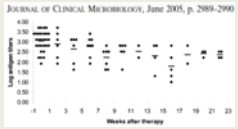
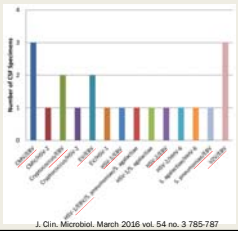
  - *S. pneumoniae*
    - 99% (1544/1556) specific but….
      - *Only 5/12 FP confirmed by alternative NAAT*
      - *9 TP, 3 FP → PPV 75%*
        - Data from package insert

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




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

## 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?