Cerebrospinal Fluid

By

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CSF Formation

- CNS covered by 3 membranes [meninges].
- Outer most membrane dura mater
- Center membrane arachnoid mater ("spider-web like")
- Inner membrane pia mater
- CSF between pia and arachnoid maters
  - subarachnoid space.
- CSF produced by ventricular capillary blood vessels
  - choroid plexuses
- CSF absorbed by arachnoid villi

CSF is a “selective” ultrafiltrate of plasma.
CSF Volumes and Function

- **Total CSF volumes:**
  - Adult 85 - 150 ml
  - Neonate 10 - 60 ml
  - Adult Rate of Formation 500 ml/day
  - Turn over = 20 mL/hour

- **Action:**
  - CNS Protection
  - CNS Waste Management
  - CNS Lubrication
  - CNS Nutrition
## Normal Values

<table>
<thead>
<tr>
<th>CSF Clarity</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Clarity</td>
<td>Clear</td>
</tr>
<tr>
<td>CSF Glucose</td>
<td>60-70% of blood glucose</td>
</tr>
<tr>
<td>CSF Glucose (Adult)</td>
<td>40-70 mg/dl</td>
</tr>
<tr>
<td>CSF Glucose (Child)</td>
<td>60-80 mg/dl</td>
</tr>
<tr>
<td>CSF WBC count</td>
<td>0-5 Leukocytes/mm³</td>
</tr>
<tr>
<td>CSF RBC count</td>
<td>&lt;1 RBC/mm³</td>
</tr>
<tr>
<td>CSF Protein</td>
<td>15-45 mg/dl</td>
</tr>
<tr>
<td>CSF Culture</td>
<td>Sterile</td>
</tr>
</tbody>
</table>
CSF Collection Indications

- Meningeal infection
- Subarachnoid hemorrhage
- Malignancy
- Neurological disease

“Fever of Unknown Origin” = FUO
I- Collection of specimens

CSF obtained by:

- Lumber puncture
- Ventricular aspirate
- Subdural tap
- Aspiration from ventricular shunt
Lumber puncture

CSF is collected by lumbar puncture between third, fourth, fifth lumbar vertebrae. The collection should only be performed by a physician.

Properly label the CSF transport sample tubes with complete patient identification and collection date.

Must be collected aseptically using acceptable lumber puncture technique which has to be non-traumatic.

The first drops must be discarded to avoid any contaminants.

Collect 3.0 - 4.0 ml C.S.F into the sterile vials.
Aspiration from ventricular catheter
3 sterile dry screw capped containers

- label one **No.1** – used for chemical and serologic test (tube is frozen).
- The other **No.2** – used for microbiology lab (room temp.)
- The third **No.3** – used for hematology (cell count) (refrigerated)

A serum glucose analysis should be done simultaneously.

- **Immediately** deliver the samples
CSF Collection

Generally, up to 20 ml can be taken from an adult, if pressure is normal (50-180 mm Hg); The sample is placed into sterile tubes, labeled 1, 2, 3 etc.

Microbiology
Chemistry
Hematology

Small volumes – sample to micro 1st (sterility issue)

Transport to Lab STAT – store at temperature to preserve analyte/constituent of interest.
Opening Pressure

- Normal opening pressure in adults is 90~180mmHg, 10~100mmHg in children.
- Measured by attaching a manometer to the hub of the needle

**Elevated pressure in:**
- Congestive heart failure
- Meningitis
- Cerebral edema
- Mass lesion

**Decreased pressure in**
- Dehydration
- Circulatory collapse
- CSF leakage
Measuring opening pressure

Source: http://www.emedicinehealth.com
Collecting CSF into sterile tubes
II- Recording

Label each tube with:

- Patient’s first and last name
- Date and time of collection
- Specimen source (CSF and i.e. lumbar, shunt, EVD)
- Tube identification number (1, 2, 3, 4) indicating order of collection

Record immediate results:

- Appearance of CSF
- Pressure of CSF
III- Handling & Transport

- Specimens before treatment ideally
- CSF must be **processed** within 1 hour of collection
- **Do not refrigerate**
- Only put CSF in an incubator if the temperature is $< 15^\circ$C and it cannot be taken to the lab quickly.

Most of CSF pathogens are very fastidious
Trans-Isolate medium

• A diphasic medium for the transport of primary cultures of cerebrospinal fluids from patients with bacterial meningitis.

• It consists of a charcoal-starch agar slant and soybean-casein digest-gelatin broth.

• In the laboratory, this medium supported the growth and survival of *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* for at least 3 months.
A delay in examining CSF:

- Reduces the chances of isolating pathogens
- Lower cell count due to WBCs being lysed
- Falsely low glucose value due to glycolysis
Criteria for rejection

- Sample improperly labelled.
- Not freshly collected
- Traumatic sample.
# Causative Infectious Agents of Meningitis

## A. Bacteria

<table>
<thead>
<tr>
<th>Common organisms</th>
<th>Uncommon organisms</th>
<th>Uncommon organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilus influenzae</td>
<td>Streptococcus pneumoniae</td>
<td>Streptococcus agalactiae</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>Escherichia coli</td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Staphylococcus aureus</td>
<td>Mima-Herellea</td>
</tr>
</tbody>
</table>

## B. Mycobacterium tuberculosis

| N. meningitidis | H. capsulatum | B. dermatitidis | S. schenckii |

## D. Viruses

<table>
<thead>
<tr>
<th>Enteroviruses (echoviruses, coxsackievirus, polioviruses)</th>
<th>Arboviruses</th>
<th>Herpes simplex, types 1 &amp; 2</th>
<th>Lymphocytic choriomeningitis virus</th>
<th>Others (rare): adenoviruses, rhinoviruses</th>
</tr>
</thead>
</table>

## E. Parasites

| Naegleria fowleri | Acanthamoeba | Angiostrongylus cantonensis |

## F. Spirochetes

| B. burgdorferi | T. pallidum |

## C. Fungi/Yeast

| Aspergillus | Cryptococcus neoformans | Candida species |
Possible pathogens according to age groups

Neonates

Infants
- *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*

Children
- *N. meningitidis*, *S. pneumoniae*

Adults
- *S. pneumoniae*, *N. meningitidis*, *Mycobacteria*, *Cryptococci*

Elderly
- As in neonates
IV- Processing of CSF samples

- CSF must always be considered as a priority specimen that requires prompt attention by the laboratory staff.

Laboratory tests on CSF

- Gross exam
- Cell Counts + Diffs
- Glucose [60-70% plasma]
- Protein [15 - 40 mg/dL]
- Stains [Gram, Acid Fast, India ink]
- Cultures
- Latex Agglutination
- Polymerase Chain Reaction
1. Macroscopic (Gross) Examination

- Color & Clarity
- Normal CSF appearance is crystal clear and colorless

- Pathological processes can cause fluid to appear cloudy, turbid, bloody, viscous, or clotted.
  - **PLEOCYTOSIS** – increased CSF cell numbers
    - WBC > 200 cells/μL
    - RBC > 400 cells/μL
# Clinical Significance of CSF Gross Appearance

<table>
<thead>
<tr>
<th>Gross Appearance</th>
<th>Cause</th>
<th>Major Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal clear</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Smoky</td>
<td>RBCs</td>
<td>Hemorrhage (early, before RBC lysis)</td>
</tr>
<tr>
<td>Cloudy, Turbid</td>
<td>WBCs</td>
<td>Traumatic tap</td>
</tr>
<tr>
<td></td>
<td>Microorganisms</td>
<td>Meningitis</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Fatty emulsion</td>
<td>Subdural fat</td>
<td>Disorders that affect blood-brain barrier</td>
</tr>
<tr>
<td>Clot and pedicle formation</td>
<td>Increased fibrinogen</td>
<td>Production of IgG within CNS</td>
</tr>
<tr>
<td>Bloody</td>
<td>RBCs</td>
<td>Aspirated during lumbar puncture</td>
</tr>
<tr>
<td>Xanthochromic</td>
<td>Hemoglobin</td>
<td>Traumatic tap</td>
</tr>
<tr>
<td></td>
<td>Bilirubin</td>
<td>Subarachnoid block (Froin’s syndrome)</td>
</tr>
<tr>
<td></td>
<td>Merthiolate</td>
<td>Suppurative meningitis</td>
</tr>
<tr>
<td></td>
<td>Carotene</td>
<td>Tuberculous meningitis</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>Hemorrhage</td>
</tr>
<tr>
<td></td>
<td>Melanin</td>
<td>Old hemorrhage</td>
</tr>
<tr>
<td>Oily</td>
<td>X-ray material</td>
<td>Lysed cells from traumatic tap</td>
</tr>
<tr>
<td>Greenish tinge</td>
<td>Myeloperoxidase</td>
<td>RBC breakdown</td>
</tr>
<tr>
<td>Viscous</td>
<td>Capsular polysaccharide</td>
<td>Elevated serum bilirubin</td>
</tr>
<tr>
<td></td>
<td>Mucus</td>
<td>Contamination</td>
</tr>
<tr>
<td></td>
<td>Liquid nucleus pulposus</td>
<td>Increased serum levels (dietary)</td>
</tr>
<tr>
<td>Fat globules</td>
<td>Fat</td>
<td>See above</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meningeal malignant melanoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purulent fluid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cryptococcosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metastatic mucin-producing carcinomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Needle injury to annulus fibrosus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat embolism</td>
</tr>
</tbody>
</table>
Bloody or blood-tinged indicates possible:
- Bloody tap
- Subarachnoid or cerebral hemorrhage
- At least 400 RBCs/µl must be present before the CSF is visibly bloody

Turbid CSF usually indicates the presence of:
- WBCs, >200 WCCs
- RBCs, >400 RBCs
- Bacteria, or
- Other microorganisms
Yellow color indicates possible:
- Previous subarachnoid bleeding
- Severe jaundice
- Large amounts of protein (>150 mg/dl)

Clots in c.s.f. indicates a high protein concentration with increased fibrinogen:
- pyogenic meningitis
- spinal constriction
Traumatic Tap or CNS Hemorrhage

- ~20% of LPs result in bloody specimens.
- Pink-red CSF usually indicates the presence of blood.
- It is extremely important to identify the source of the blood.
Clues Useful in Differentiating Traumatic Tap from CNS Hemorrhage

- Traumatic tap demonstrates
  - maximum amount of blood in first sample tube with progressive decrease in subsequent sample tubes
  - the sample from a CNS hemorrhage demonstrates blood evenly mixed in all collection tubes
- After CSF sample centrifugation,
  - the supernatant from a traumatic tap is clear
  - The supernatant is “xanthochromic” from a hemorrhage
- A very bloody tap may demonstrate blood clots in the CSF sample, while clots are not usually associated with CNS hemorrhage.
Xanthochromia

Defined as a **pink**, **orange** or **yellow** color of CSF supernatant.

P = oxyhemoglobin
Y = bilirubin
O = combination

Subarachnoid and intracerebral hemorrhage
Traumatic tap  **2-3 days post tap**
Jaundice
Elevated protein level (> 150 mg/dL)
Premature infants with immature blood, CSF barrier, and elevated bilirubin
Hypercarotenemia
Meningeal malignant melanoma
Traumatic Tap or Not

- **Traumatic**
  - More blood in 1\textsuperscript{st} tube
    - Blood concentration decreases in subsequent tubes
  - Clear supernatant after centrifugation
  - Phagocytized RBCs by macrophages
  - Hematoidin Bodies in macrophages

2. Cell Counts

- Cell counts
  - Total
  - Leukocyte
  - RBC
- Differential

![Image of blood cells]

Examination of body fluid

- Clear fluid
  - Count undiluted

- Very cloudy or bloody fluid
  - Dilute* and count
  - Concentrate cells
  - Make smears

- Wright-Giemsa stain
- Wright-Giemsa stain
- Gram's stain

* Dilution:
  - 1:10 dilution on slightly cloudy fluids
  - 1:20 dilution on moderately cloudy fluids
  - 1:100 dilution on very cloudy or bloody fluids with saline

[Image: http://www.neuropat.dote.hu/jpeg/liquor/kmcarc1.jpg]
Cell Counts

- Manual counts
  - Hemocytometer
  - # cells x dilution factor / volume

- Automated Count
  - Flow cytometers
  - Coulter-based cell counter
Counting chambers

- A Fuchs-Rosenthal chamber is recommended because it has twice the depth (0.2 mm) and is more suitable for counting WBCs in c.s.f.

- When unavailable, an improved Neubauer chamber can be used.
Cell count

- Count all the white cells lying within the square and those touching the upper and right-hand center lines.
- The white cells that touch the left-hand and bottom lines are not to be counted.
- In each of the four areas, conduct the count as indicated by the "snake-like" line.
Calculation

- The depth of the counting chamber is 0.1 mm and the area counted is 4 mm$^2$ (4 squares are counted, each with an area of 1.0 mm$^2$ therefore, $4 \times 1.0 \text{ mm}^2 = \text{a total of } 4 \text{ mm}^2$).

- The volume counted is: area $\times$ depth = volume. Four mm $\times$ 0.1 mm = 0.4 mm$^3$.

- The formula is as follows:
  - No. of cells $\times$ dilution factor/volume

- Calculate the number of WBCs per mm$^3$: 
Cover glass

Cover glass support

Counting chamber
0.1mm depth
Example

Calculation factors when using 1 in 10 c.s.f. dil:

using an Improved Neubauer chamber,

- If 64 cells are counted in 4 squares:

  \[
  \text{No. of cells} \times \text{dilution factor/volume} = 64 \times 10 / 0.4 = 1600 / \text{mm}^3
  \]

  Report as 1600 X 10^6 cells/l.

Using Fuchs-Rosenthal chamber:

- If 64 cells are counted in 5 squares:

  \[
  64 \times 10 / (5 \times 0.2) = 640 / \text{mm}^3
  \]

  Report as 640 X 10^6 cells/l.
Cell Counts

- Reference range WBC count of “normal” CSF:
  - 0-5 cells/ mm³ in adult
  - 20 cells/ mm³ in newborns

- Mononuclears

- 87% of patients with bacterial meningitis will have >1,000/ mm³

- 99% will have >100/ mm³.

- <100 WBCs/ mm³ is more common in viral meningitis.
- The normal red blood cell count is 0.
- RBC count is of limited use, but can be used to correct CSF leukocyte counts of a traumatic tap CSF.
- Peripheral blood in the CSF after a “traumatic tap” will result in an artificial increase in WBCs by one WBC for every 500 to 1,000 RBCs in the CSF. This correction factor is accurate as long as the peripheral WBC count is not extremely high or low.
Differentials

- Performed on a stained* smear made from CSF.
- It is recommended that stained smears be made even when the total cell count is within normal limits.
- Count 100 cells in consecutive oil-power fields.
- Report percentage of each type of cell present.

*usually Wright’s stain
## Normal CSF Differential Cell Count

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Adults</th>
<th>Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>60% ± 20%</td>
<td>20% ± 15%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>30% ± 15%</td>
<td>70% ± 20%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2% ± 4%</td>
<td>4% ± 4%*</td>
</tr>
<tr>
<td>Neuroectodermal cells</td>
<td>Rare</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Note: Sedimentation or cytocentrifuge methods used.
Data adapted with permission from: Krieg AF, Kjeldsberg CR.\(^{10}\)

* In high-risk neonates without meningitis, the CSF may have 60% neutrophils. Data from: Sarff LD et al.\(^{13}\)
Cells Observed in CSF

CSF cytoprep, Wright-Giemsa. 1000x
A – PMNs, Lymphocytes; B – Lymphocytes; C – Monocyte.
3. Chemical Analysis of CSF

- **Protein**
  - 80% plasma derived
    - LMW
      - Transthyretin (prealbumin)
        - Albumin
      - Transferrin
      - IgG – very small amount
    - 20% intrathecal synthesis
  - Reference range
    - 15 – 45 mg/dL
    - 150 mg / dL (Neonates)

- **Glucose**
  - Need to know plasma value
    - Increased
      - Hyperglycemia
      - Traumatic tap
    - Decreased
      - Hypoglycemia
      - Meningitis
      - Tumors
  - The normal Glucose is about 60% compared to serum level.

- **Glucose**
  - Need to know plasma value
    - Increased
      - Hyperglycemia
      - Traumatic tap
    - Decreased
      - Hypoglycemia
      - Meningitis
      - Tumors
Albumin and IgG

- Albumin not made in CNS
- ALB used to address blood-brain barrier integrity
- Evaluate CSF/serum ALB index
  - Index < 9 = normal
  - 9 – 14 minimal impairment
  - > 100 = not intact barrier

- IgG sourced from inside and outside CNS
- ALB used as reference protein to ID intrathecal source of Ig
- CSF IgG index = ratio IgG_{CSF}/IgG_{serum} \times ALB_{serum}/ALB_{CSF}
  - Reference range 0.3 – 0.7
    - > 0.7 = CNS sourced
    - < 0.3 = compromised BBB
Typical CSF findings in Meningitis

### Bacterial meningitis

1. Presence of **neutrophils** in the CSF is associated with infection by *N. meningitidis, S. pneumoniae* etc.

2. CSF protein level reflects the degree of meningeal inflammation:
   - **10 X** in bacterial infections

3. CSF glucose levels:
   - **very low** in bacterial infections

### Viral meningitis

1. Presence of **lymphocytes** is associated with infection by viruses or mycobacteria.

2. CSF protein level reflects the degree of meningeal inflammation:
   - **2-3 X** in viral CNS infection

3. CSF glucose levels:
   - **normal** with viral infections
Value of LP findings – Acute bacterial meningitis.

All the true acute bacterial meningitis cases
Bedside assessment alone is very helpful.

75% of acute bacterial meningitis cases can be detected by examining for CSF cloudiness or turbidity at the bedside.
CSF Cloudiness / Turbidity

A simple test of CSF turbidity is to see if normal print can be read easily through the sample – CSF should be crystal clear.

Cloudiness usually appears at CSF WBC counts > 200x10^6 Wbc per L
CSF culture is great but if it is not available a microscope will provide nearly all you need to know.

82% of acute bacterial meningitis cases can be detected by either turbidity or a CSF white cell count and using a cut-off of >50 Wbc per μL. (>50 \times 10^6 \text{ Wbc per L})
CSF microscopy, blood and CSF glucose measures are highly sensitive.

96% of acute bacterial meningitis cases can be detected by turbidity, a CSF white cell count of >50 Wbc per μL (>50 x 10⁶ Wbc per L) or a CSF glucose to Blood glucose ratio <0.1*

* Books suggest a ratio of ~0.6, this is much too high to be useful
4. Microscopic examination

Preparation of specimen:

- If, on gross examination, the CSF is purulent (very cloudy), it can be examined immediately without centrifugation.
- In all other cases, the CSF should be centrifuged in a sterile tube.
- Remove the supernatant using a sterile Pasteur pipette, and transfer it to another tube for chemical and/or serological tests.
- Use the sediment for further microbiological tests.
Gram Smear

- Gram stain is positive in 60 to 80 % of untreated cases of bacterial meningitis and in 40 to 60 % of partially treated cases.
- A Gram smear may also provide useful information when a c.s.f. is unsuitable for cell counting or biochemical testing (e.g. when it is heavily blood stained or contains clots).
- *When bacteria and pus cells are seen in the* Gram smear, *culture the c.s.f.* There is no need to perform a cell count or measure the protein or glucose.
Several factors influence the sensitivity of Gram stain:

- The sensitivity according to the causative organism ranges from 90% in pneumococcal or staphylococcal meningitis to less than 50% in Listeria meningitis. Hyphae can occasionally be seen in Candida or other fungal meningitis cases.

- Laboratory techniques used to concentrate and stain CSF. Cytocentrifugation increases the ability to detect bacteria.

- Greater numbers of colony-forming units (CFU) per mm3 of CSF.

- The experience of laboratory personnel is very important. Up to 10 percent of initial Gram stains are misread.
Important: Advice the medical officer immediately

- If the Gram smear contains bacteria, pus cells, or yeast cells (confirmed as capsulated in India ink preparation).

**Acridine orange** stained smear to detect bacteria in c.s.f.

- When facilities for fluorescence microscopy are available, examine an acridine orange (A0) stained smear.
- Bacteria especially when few, are more easily detected in A0 smears.
- They stain bright orange and cells and debris stain green or yellow.
Acid-fast stain (Ziehl–Neelsen)

- Acid-fast staining should be done if tuberculosis is clinically suspected or the c.s.f. contains lymphocytes and the glucose concentration is low and the protein raised.
- Only 37 % of initial smears will be positive for acid-fast bacilli. This result can be increased to 87 % if four smears are done.
- Sensitivity also can be increased by examining the CSF sediment.

**Fluorochrome smear to detect *M. tuberculosis* in c.s.f.**

- When facilities for fluorescence microscopy are available, examination of an auramine stained smear is a more sensitive
India ink preparation

- When cryptococcal meningitis is clinically suspected, e.g. patient with HIV disease, or when yeast cells are detected when performing a cell count or examining a Gram smear.

- Cryptococcus may be identified up to 50% of the time on an India ink preparation.

- Look for oval or round cells, some showing budding, irregular in size, measuring 2–10 m in diameter and surrounded by a large unstained capsule.

- A tap-water control should always be done to ensure that the India ink is not contaminated.
5. Culture

- Cultures done on blood agar and chocolate agar remain the gold standards for diagnosing bacterial meningitis.
- Immediately after centrifugation of the CSF and the removal of some of the debris for the Gram film, the remainder of the deposit should be seeded heavily into culture media.
  - Blood agar
  - MacConkey agar
  - Chocolate agar: with CO2
- Incubated aerobically and anaerobically
- All media should be incubated for 3 days, with daily inspections.
- Antibiotic treatment prior to lumbar puncture can decrease the sensitivity of culture,
Culture

- When **tuberculous meningitis** is suspected, at least three tubes of Löwenstein–Jensen medium should be inoculated with a drop of the sediment and incubated for 6 weeks.
- **Mycobacterium tuberculosis** is best grown using multiple large volume samples of CSF.
- At least 15 mL and preferably 40 to 50 mL of CSF are recommended.
- Culture is positive 56 % of the time on the first sample, and improved to 83 % of the time if four separate samples are cultured.
- These cultures often take up to six weeks for positive identification.
When *Cryptococcus neoformans* is suspected, either from the India ink preparation or on clinical grounds, the sediment should be inoculated on two tubes of Sabouraud dextrose agar, and incubated at 35 °C and 37 °C for up to 1 month.

Fungal cultures are positive in more than 95 % of Cryptococcus neoformans cases and in 66 % of candidal meningitis cases.

Other fungi are less likely to be culture positive.

*C. neoformans* also grows on the blood agar plate, which should be incubated at 35oC for 1 week, if indicated.
- **Blood culture** should be done with csf culture.
- Bacterial meningitis is often ass.wth bacteraemia and the causative organism is sometimes isolated from blood when csf culture is negative.
LP changes after antibiotics.

- Prior antibiotic use reduces the sensitivity of CSF gram stain by 20% and CSF culture by 30%.
- It takes **24-36 hours** of therapy before >90% of CSF cultures will be sterile.
- It takes **2-3 days of therapy** to change the WBC cell count of CSF in true bacterial meningitis.
- *Prior treatment is no excuse for not doing an LP!*
N. meningitidis
S. pneumoniae
H. influenzae
Cryptococcus neoformans. India ink staining of CSF
6. Serologic testing

- Latex agglutination (LA) allows rapid detection of bacterial antigens in CSF.

- Tests are available to detect *N. meningitidis* groups A, B, C, Y and W135 (*Group B* reagent cross-reacts with *E. coli K1* antigen), *H. influenzae* type b, *S. pneumoniae*, and *S. agalactiae*.

- Sensitivity varies greatly between bacteria. LA for *Haemophilus influenzae* has a sensitivity of 60 to 100 %, but is much lower for other bacteria.
The specificity for LA is very low. However, LA can be useful in partially treated meningitis cases where cultures may not yield an organism.

Because false positives lead to unnecessary treatment, LA is not routinely used today. Some experts suggest using LA in cases of suspected bacterial meningitis if the initial Gram stain and bacterial culture are negative after 48 hours.

Crypto antigen screen detects C. neoformans; + in 90-95% of pts with crypto meningitis
7. Polymerase Chain Reaction

- PCR has been a great advance in the diagnosis of meningitis. PCR has high sensitivity and specificity for many infections of the CNS, is fast, and can be done with small volumes of CSF.

- Although testing is expensive, there is a potential for cost savings by decreasing overall diagnostic testing and intervention.

- PCR has been especially useful in the diagnosis of viral meningitis. PCR of the CSF has a sensitivity of 95 to 100 %, and a specificity of 100 % for herpes simplex virus type 1, Epstein-Barr virus, and enterovirus.
- PCR has a sensitivity of 54 to 100 % and a specificity of 94 to 100 % for tuberculous meningitis, and could replace acid-fast bacillus smear and culture as the test of choice.

- PCR is sensitive for acute neurosyphilis but not for more chronic forms.

- PCR also is being studied as a diagnostic tool for bacterial meningitis and other infections of the CNS.
Summary of the Examination of C.S.F.

**Day 1**

1. **Report Appearance**
   - Describe whether c.s.f.
     - Clear, slightly turbid, cloudy, purulent
     - Contains blood
     - Contains clots

2. **Test c.s.f.**

   - **Purulent or cloudy c.s.f.**
     - Suspect pyogenic bacterial meningitis
       - **Gram smear**
         - Report:
           - Number of pus cells
           - Bacteria
       - **Culture c.s.f.**
         - Blood agar and chocolate agar.
           - Incubate in CO₂
         - If neonate:
           - Also MacConkey agar.
           - Incubate aerobically

   - **Slightly turbid or clear c.s.f.**
     - Perform cell count
       - Note whether pus cells or lymphs
     - **PUS CELLS**
       - **Gram smear**
         - See opposite
     - **Culture c.s.f.**
       - See opposite
     - **LYMPHS**
       - Measure protein
       - Measure glucose
       - **Zn:** For AFB
       - **India Ink:** For encapsulated yeasts
     - **ADDITIONAL TESTS**
       - **Wet preparation:** For motile amoebae
         - **Wet preparation and Giemsa smear:** For trypanosomes and morula cells
Day 2 and Onwards

3 Examine and Report Cultures

- Chocolate agar and blood agar cultures
  Look particularly for:
  * N. meningitidis
  * S. pneumoniae
  * H. influenzae (chocolate agar)

- MacConkey agar culture
  Look especially for bacteria that cause neonatal meningitis

ADDITIONAL
- Perform antimicrobial susceptibility tests as indicated
- *Beta*-lactamase test.
- *H. influenzae* isolates
THANK YOU