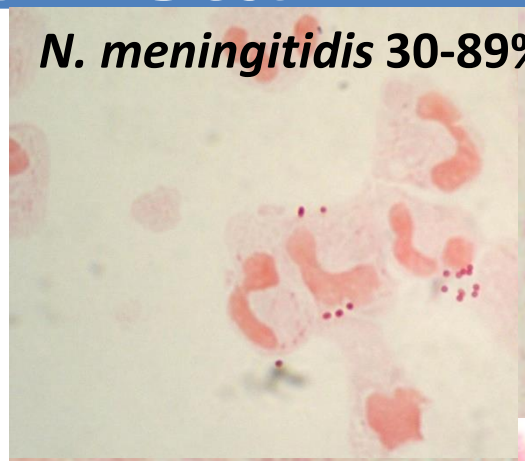
Detection of bacterial meningitis by Gram stain

- **Fairly high sensitivity and excellent specificity in untreated patient**
  - **Sensitivity**
    - ***L. monocytogenes* 10-35%**
    - ***H. influenzae, S. suis* 50%**
- **Cytospin preparation enhance detection 100X**
- **1 hr T-A-T**

Latex agglutination: 15 min sensitivity
- *H. influenzae* 78-100%
- *S. pneumoniae* 59-100%
- *N. meningitidis* 22-93%

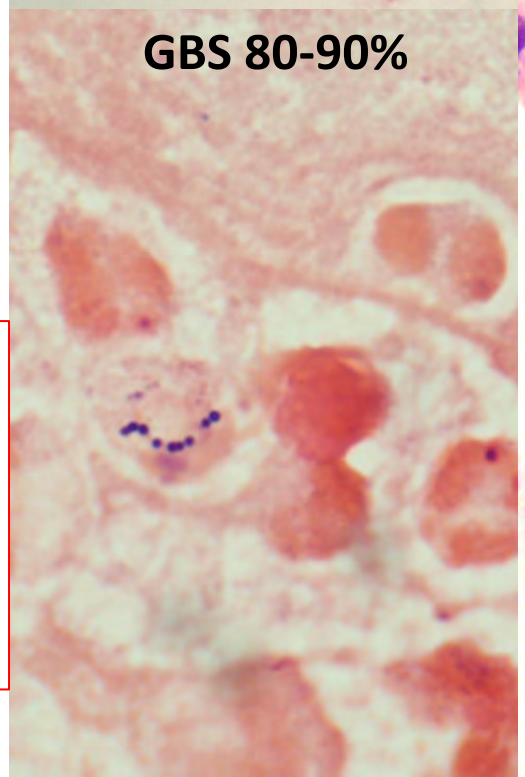
Concern about false positive
Lower sensitivity in postantibiotics
→ May not help/increase diagnostic yields and decision of therapy
→ Not advised per ECSMID2016, IDSA2004

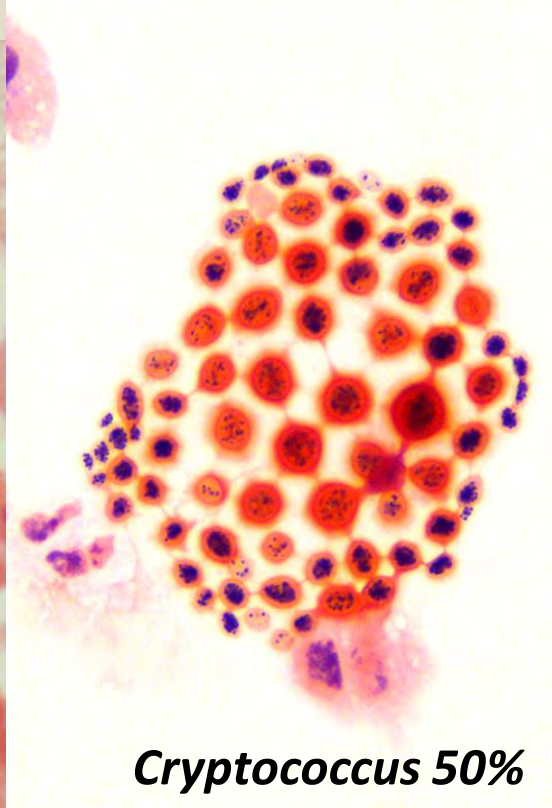
***N. meningitidis* 30-89%**



***S. pneumoniae* 69-93%**

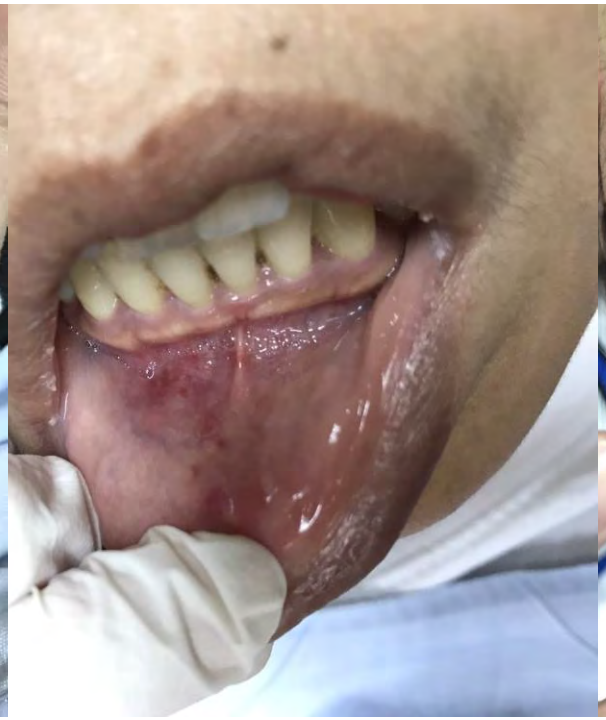
GBS 80-90%

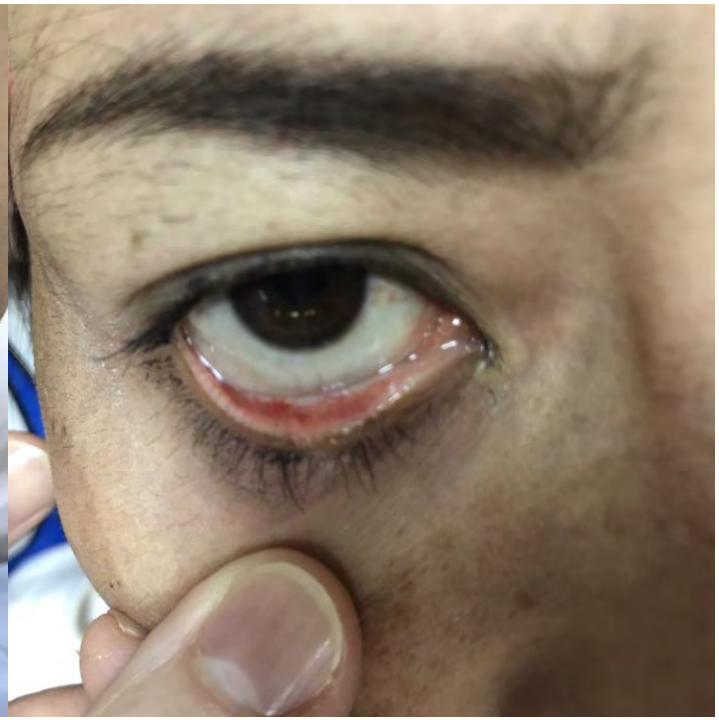




***Cryptococcus* 50%**

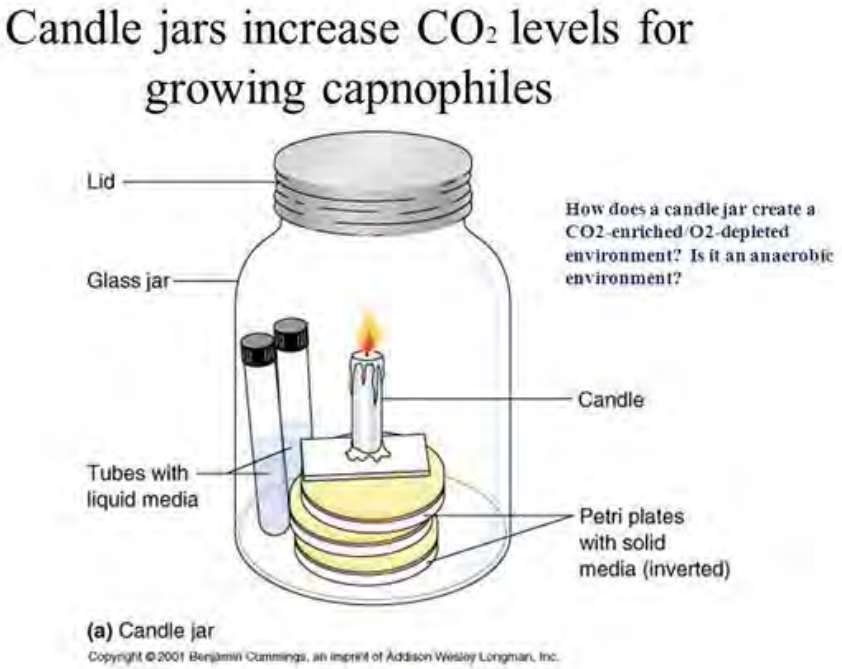
A 35-year-old with oligoarthricular arthritis and fever

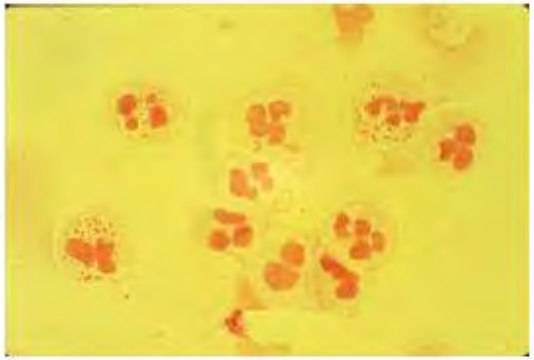




Culture of gonococci

- **Blood cultures: inhibited by SPS**
- **Chocolate agar for sterile fluid**
- **Modified Thayer Martin Agar (vancomycin, colistin, nystatin, trimethoprim) for nonsterile**



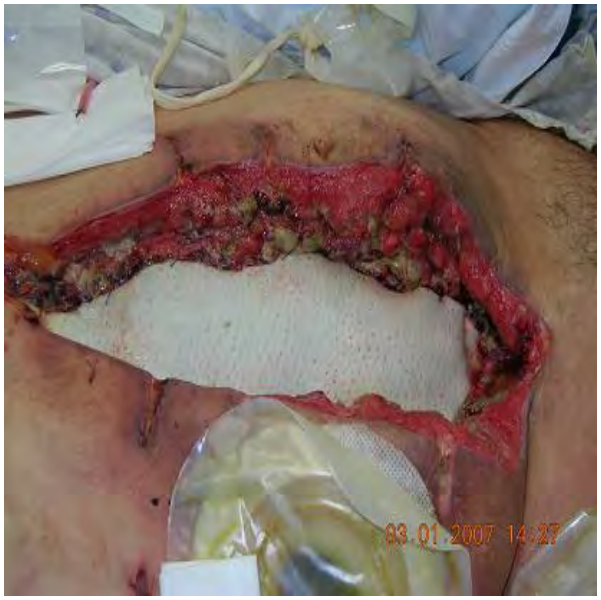


Gram stain for male urethral samples

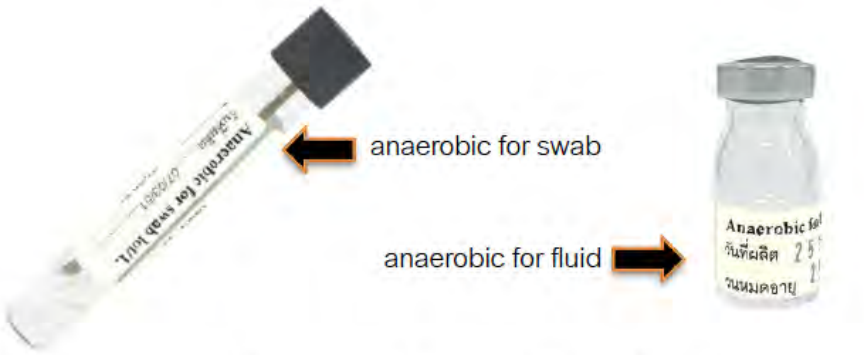
Urine culture

- **Culture** is **gold standard**
- **Collection** is important
  - **Morning voided, midstream (clean-catch)**
    - **Women**
      - **Contamination: midstream with cleansing = without cleansing**
      - **Diagnostic accuracy: midstream with cleansing = without cleansing**
    - **Men**
      - **Contamination: midstream with cleansing < first-void; with cleansing= without cleansing**
      - **Diagnostic accuracy: midstream with cleansing = straight catheterization or suprapubic aspiration**
    - **Children**
      - **Contamination: midstream with cleansing < without cleansing, sterile urine bag collection and diaper collection**
      - **Diagnostic accuracy: vary but midstream with cleansing > sterile urine bag collection**
- **Do not** collect from urine bag, **Do not** submit Foley cath tip
- **Transport** immediately <2h or refrigerate not over 24h
- **Interpret** carefully
  - Significant bacteriuria

Wound Culture



- **Send Blob, Not Swab**
  - swabs pick up extraneous microbes, hold extremely small volumes of the specimen (0.05 mL), and make it difficult to get organisms away from the swab fibers, and the inoculum from the swab is often not uniform
- **Deep tissue, No superficial swab**
- **Direct specimen, not from reservoir**



Anaerobic transport medium

Modified Cary-Blair medium

Rejection criteria for anaerobic culture

- **Superficial wound and abscess**
- **Gastric intestinal fluid**
- **Colocutaneous fistula**
- **Stool**
- **Vaginal, cervical, urethral swab**
- **Sputum, throat, tonsillar, gingival, NP, nasal swab**
- **Voided, Foley and catheterized urine**

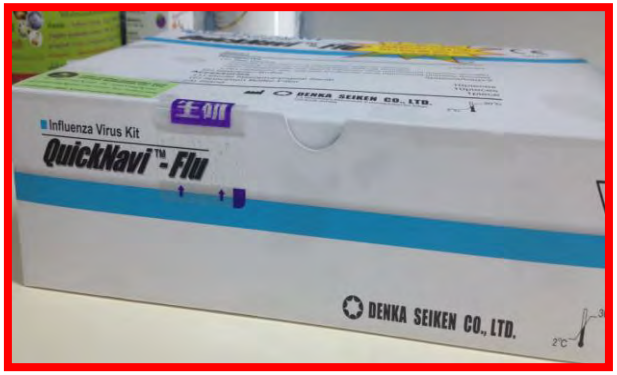
Send to the lab immediately

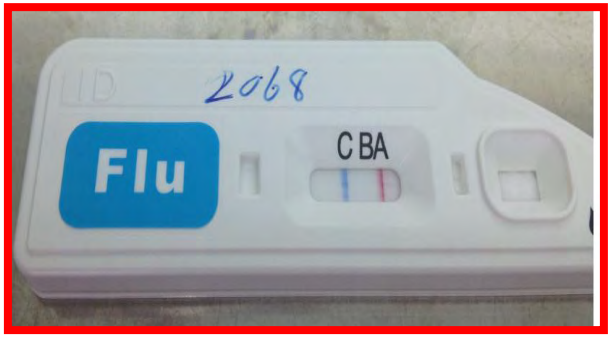
Collection, transportation and processing of specimen for fungal culture

- Transport in leak-probe sterile container
- Never used anaerobic transport media
- Transport at room temperature within 2 h, if longer usually not need to be refrigerated unless overgrowth of bacteria is concerned
- Never refrigerated sterile body fluids or if dermatophyte, *Mucorales* or Pythium insidiosum is suspected
- With one exception, fungi present in tissue are best recovered when the tissue is minced, not ground. In particular, for the mucoraceous molds, mincing is critical for the recovery of organisms
- When *H. capsulatum* is suspected, the tissue should be ground or homogenized
- *Malassezia* and lipid: yeast seen on Gram stain but no growth

McGowan KL. MCM 11thEd. 2015. Chapter 114.

Rapid Test for Flu





- Rapid turn around time
  - ~15-30 min
- Relatively poor sensitivity
  - 10-70%, vary by viral load
  - Most negative rapid antigen test results should be confirmed by another method
  - High specificity (90-95%)
- Do not subtype
- Replaced by NAAT

Culture: 3-10 d

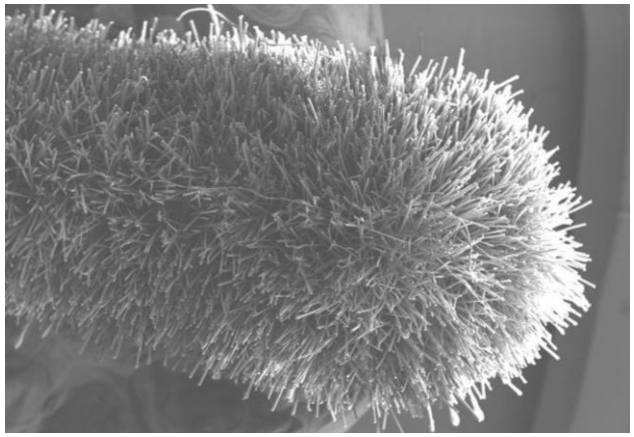
Lancet Infect Dis 2013;13:647-9

J Virol Methods 2013;187:51-6..

Specimen collection

- **Site of viral replication**
  - **NP region > throat swab; combining may improve virus detection**
  - **Throat swab: Adv, avian influenza**
  - **LRTI: Sputum, tracheal aspirates, BAL**
    - **False negative NP test 10-35% of patients with influenza pneumonia**
  - **SARS- MERS-CoV: stool may provide additional yield**

- **Flocked swab > Calcium alginate swabs or swabs with wood shaft (interfere with NAATs)**
- **Viral transport medium, refrigerated – avoid freeze and thaw**
- **False-negative results can occur due to improper specimen collection or handling**
- **A negative result can also occur when the patient is no longer shedding detectable virus, or at least at the site of collection**



Stellrecht KA. Molecular testing for respiratory viruses. In Coleman and Tsongalis, editors. Diagnostic Molecular Pathology: A guide to applied molecular testing. Academic Press. 2017

Timing of Disease/Shedding

- Generally peak shedding occurs on the first or second day of acute illness and generally declines substantially after 4 days
- Varies with virus, patient age, severity of illness, comorbidities, and immune status
  - Often hospitalized patients, particularly with LRTI, have higher viral titers and shed virus longer, drug-resistant influenza
  - NAATs detect viral targets for a longer duration than other test methods and it is not unusual to detect viral nucleic acid a couple of weeks after infection, albeit the mean duration is generally 6-14 days
  - Of the Paramyxoviruses, HMPV may be shedded relatively shorter while PIV3 maybe longer
  - AdVs and picornaviruses, exhibit prolonged shedding in both asymptomatic and symptomatic patients, which can be a diagnostic conundrum
  - Severely immunocompromised: maybe months after infection

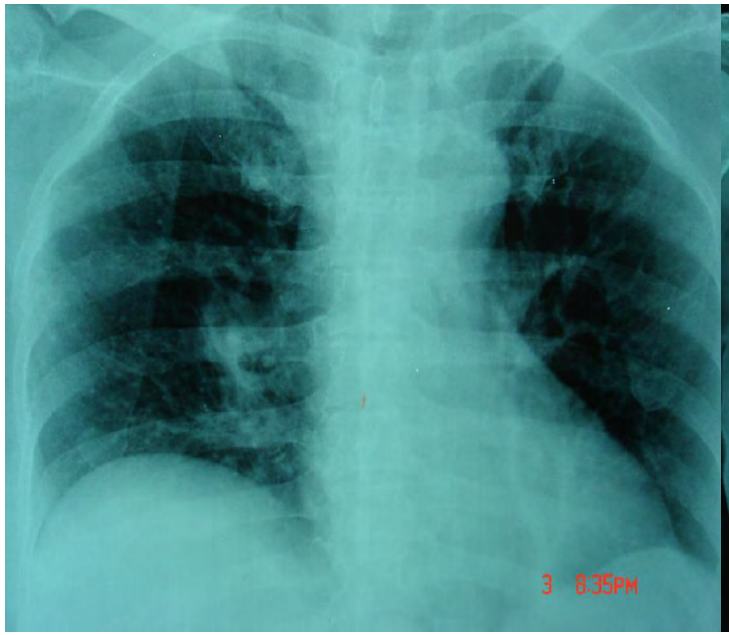
Stellrecht KA. Molecular testing for respiratory viruses. In Coleman and Tsongalis, editors. Diagnostic Molecular Pathology: A guide to applied molecular testing. Academic Press. 2017

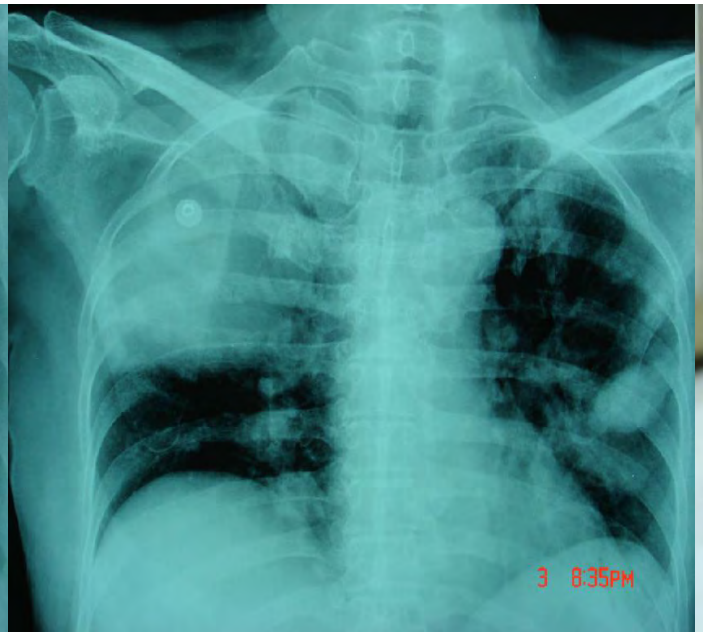
Bedside testing is still useful

Why bother?

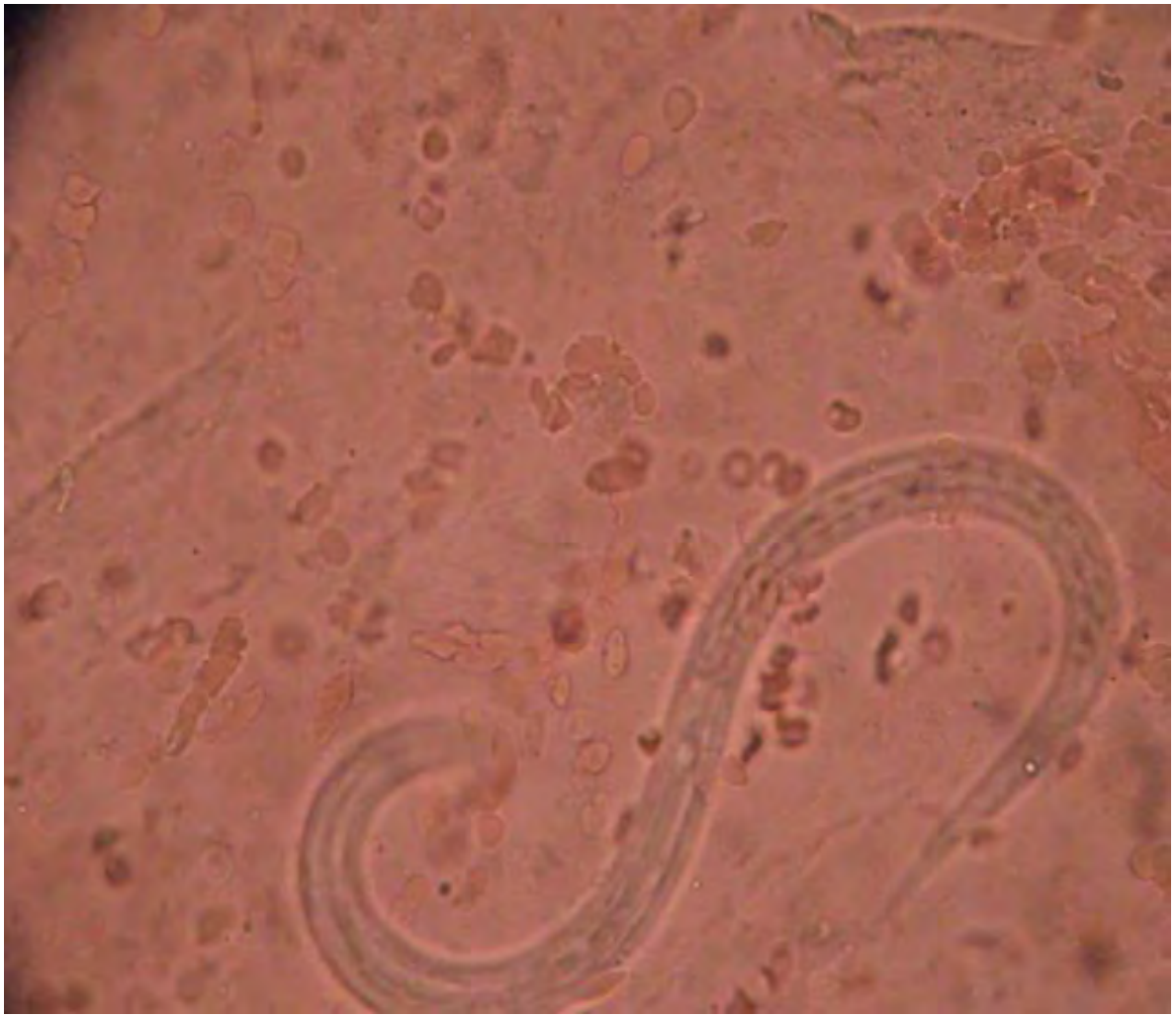
- Rapid and simple
- Prelim information to make a decision to or not to treat/empirical therapy
  - How broad is too broad?
  - No drug is safe drug
  - CSF Gram stain
- Infection is an interaction between host, organisms (type and abundance) and environment
- To access quality of the specimens
- Antimicrobial stewardships
- Labs will interpret smears unbiasedly
- No test is 100% accurate

A 20-year-old female with AML S/P induction chemotherapy day 14

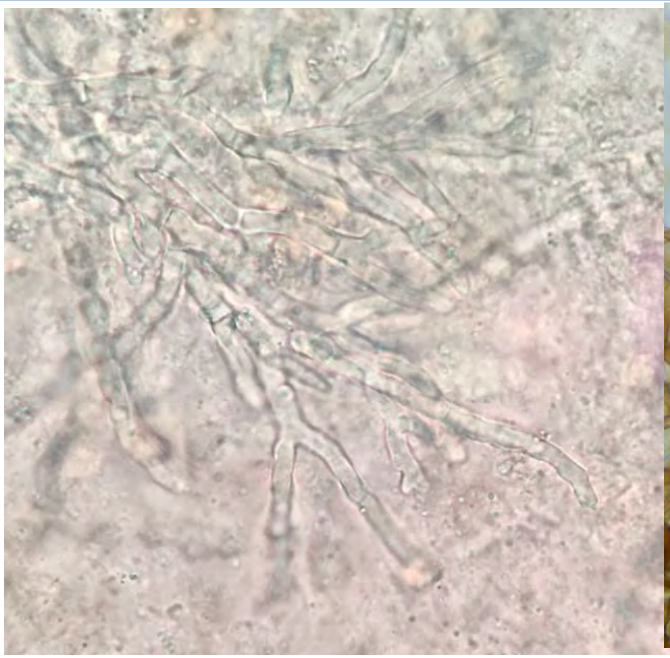




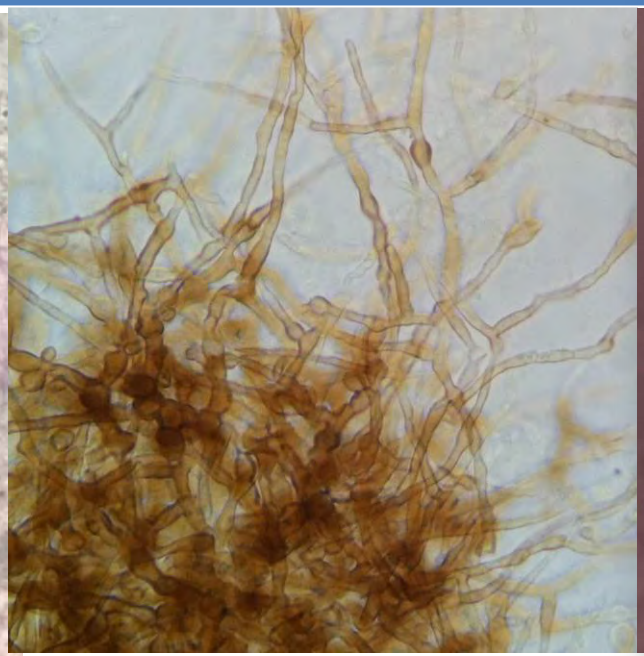
Wet Preparation



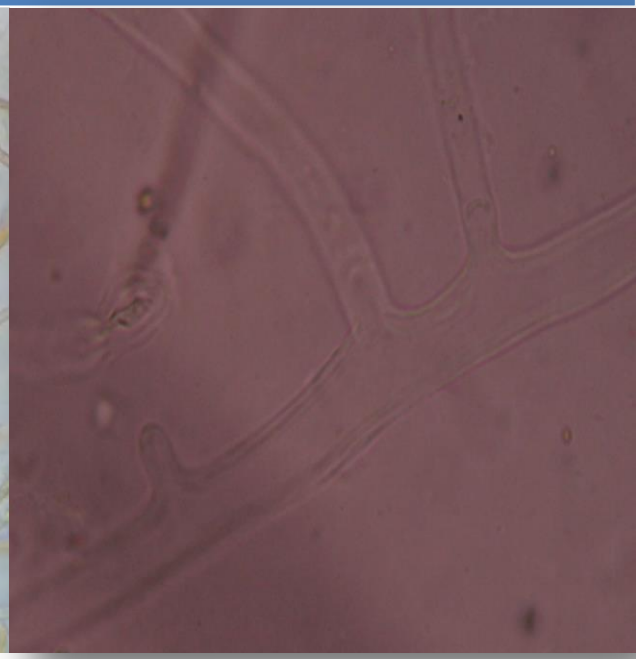
Fresh smear/ wet prep



***Aspergillus*: hyaline, septate hyphae with dichotomous branching**
DDx. Fusarium, Paecilomyces, Scedosporium, Penicillium



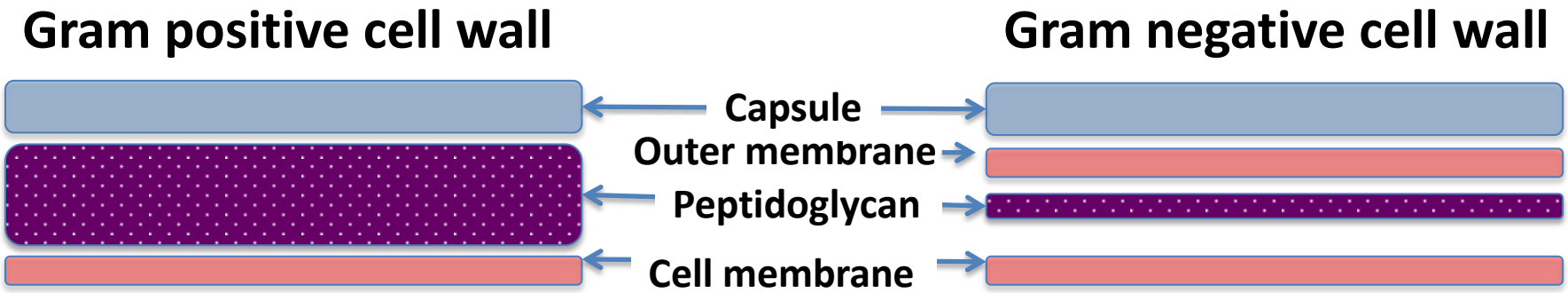
Dematiaceous (black) mould

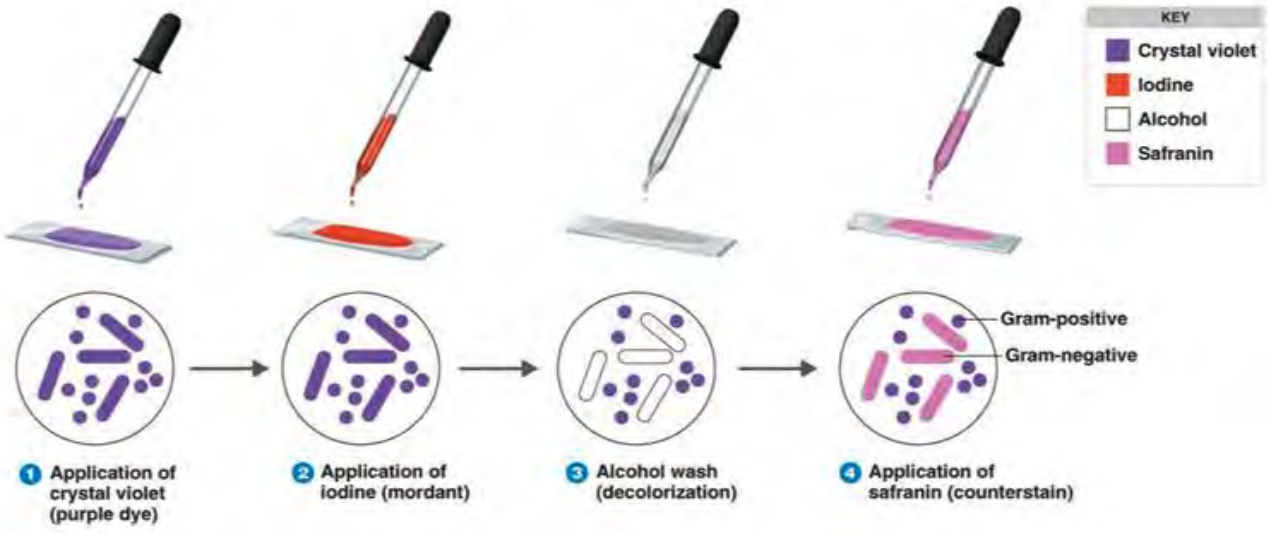


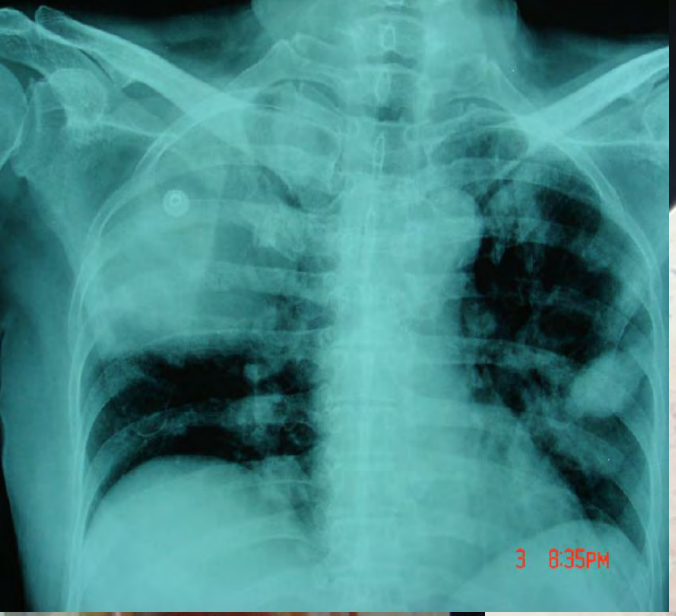
Zygomycosis: hyaline, broad, ribbony, pauci-septate hyphae with right – angle branching

- **Simple**
- **Screening with low objectives**
- **Moving organisms, moulds**
- **KOH, Lugol's iodine, India ink**

Bacterial cell envelope and Gram stain procedure



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