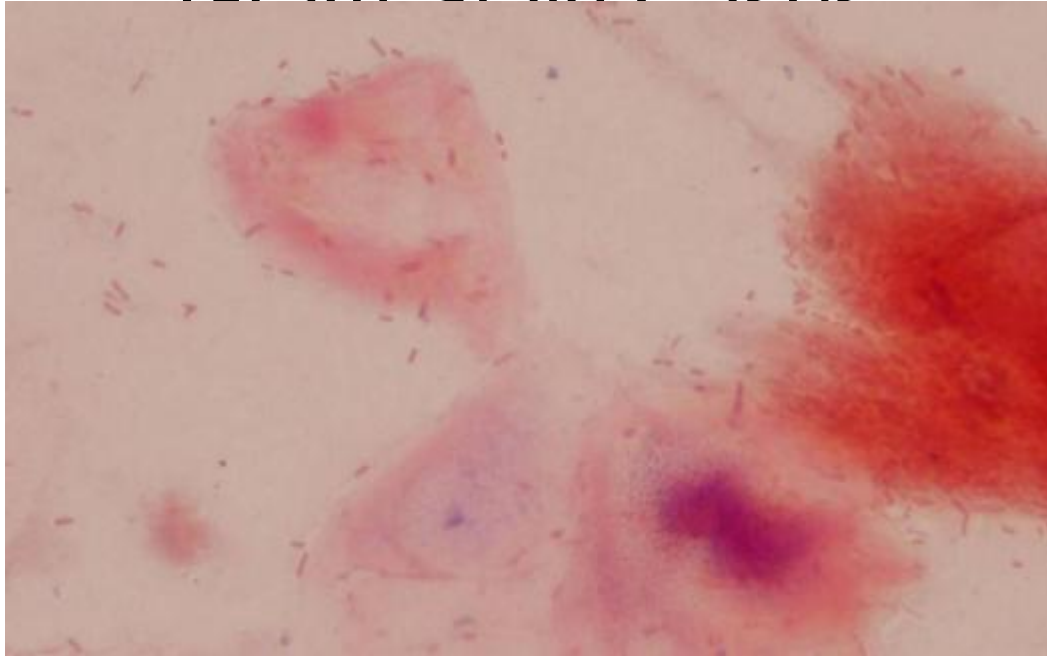


# Making a Real Difference in Real Time: Gram Stain Cases



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# Objectives

- Describe the real-time clinical utility of the Gram stain on specimens from various body sites
- Explain simple improvements that assure excellent Gram stains
- Evaluate common Gram stain technical and interpretive errors, and discuss specific cases where Gram stains made an immediate impact (either positive or negative) on patient care

Fine print disclosures: I have not taken money, pizza, pens, or note pads from any vendors in the last three years.  
My lab has done funded *C. difficile* research for Meridian Biosciences Inc.  
This research is not pertinent to this presentation.

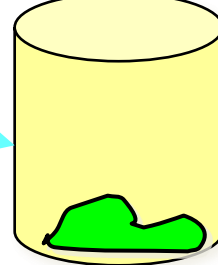
# Three keys

- The gram stain is still the best, fastest and cheapest STAT microbiology test
- Body site and patient history give the biggest hints as to what we might see
- Having the patient **past** history is nice, but we are in the patient **present**, (they are hurting) and if we do the gram stain right, we can make a positive outcome in the patient **future!**

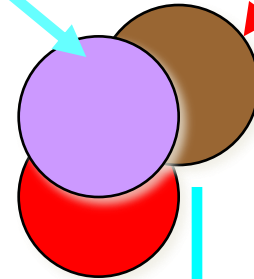


Doug

Specimen

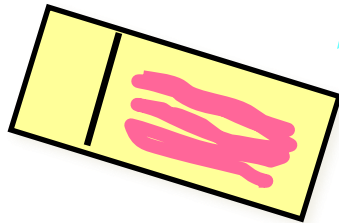


Bug



Culture,  
24 hours

Drug



Gram stain,  
30 minutes



Rapid flu test, 20 minutes  
Real time PCR for some  
Organisms, one hour



Antibiotics susceptibility  
8 to 24 more hours

Detect, identify, eliminate. A progression of actionable information leading to treatment and cure!

# Gram stain clinical impact

- If we are doing them slow, like once per shift, does it matter?
- If the doctor is going to administer empiric antibiotics, does it matter?
  - Normally sterile body site results have most clinical impact
- “No organisms seen” can actually be helpful
- May diagnose infections and guide appropriate therapy real-time
  - GNR versus GPC versus yeast versus fungi

This both bothers and encourages  
me!

## Detection and Treatment of Bloodstream Infection: Laboratory Reporting and Antimicrobial Management

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**We analyzed antimicrobial use in 509 episodes of clinically significant bloodstream infection to assess the impact that microbiology laboratory reporting had on antimicrobial management. Most therapy interventions occurred at the time of phlebotomy and after notification of Gram stain results by telephone. Release of antimicrobial susceptibility data had the least impact on antimicrobial management.**

## Gram stains led to more antibiotic interventions than AST results

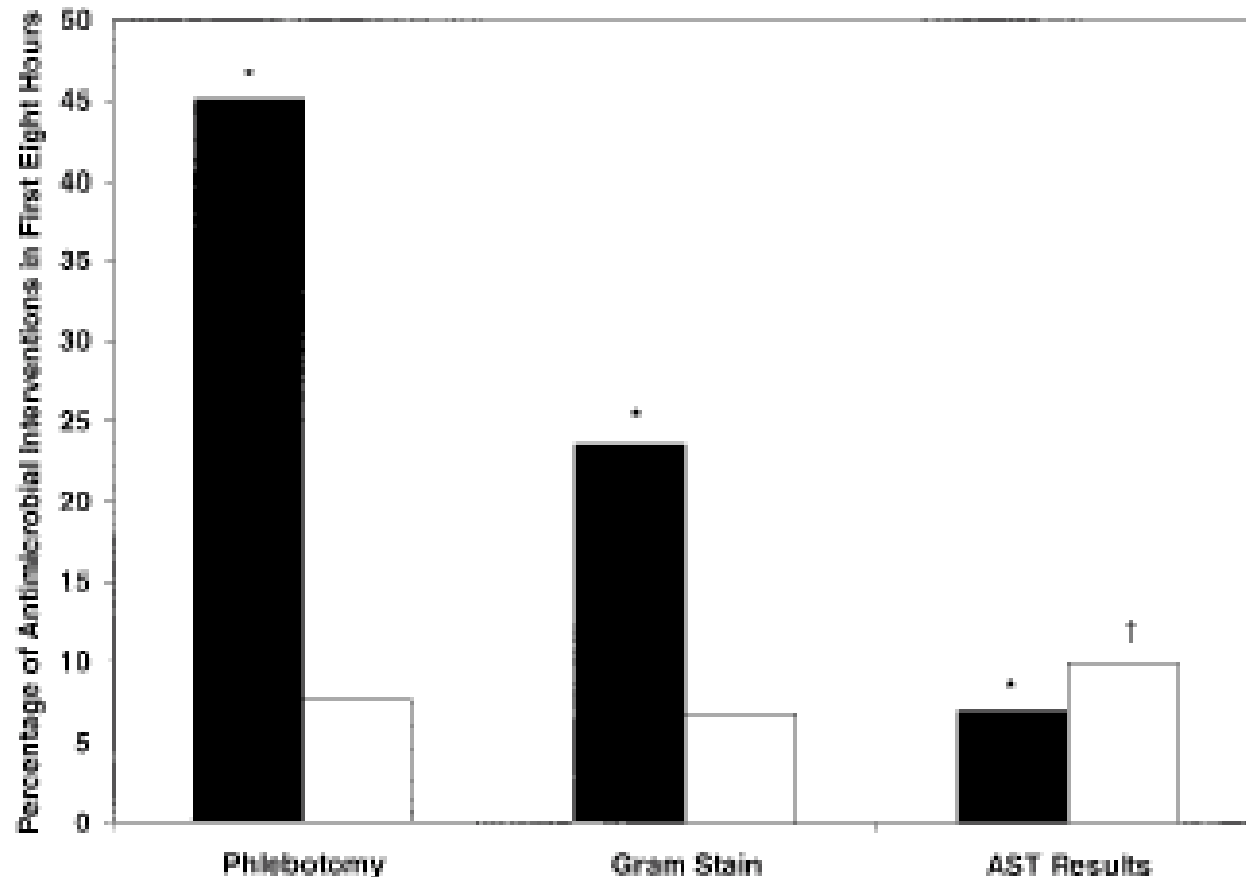


FIG. 1. Percentage of all antimicrobial interventions occurring within the first 8 h (initiations, solid bars; discontinuations, open bars) after each event of interest. \*,  $P < 0.001$  for differences noted in therapy initiations. †,  $P < 0.05$  for differences noted in therapy discontinuations.

# Consistency is lacking

- Collection: specimen versus swab of specimen
- Specimen: liquid, viscous, solid
- Technique
- Turn around time
  - sometimes the culture is read before the Gram stain
- How much time is spent examining?
- Reporting: rare, few, mod, many versus 1+, 2+
- Not as sensitive as culture, but **FASTER!**

# Four factors for success:

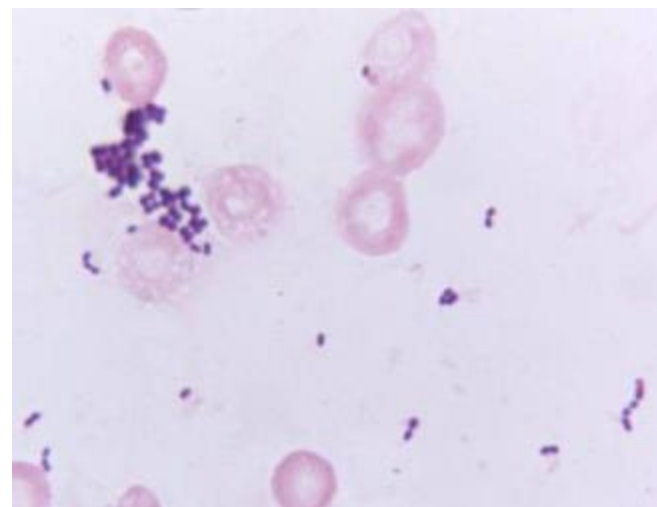
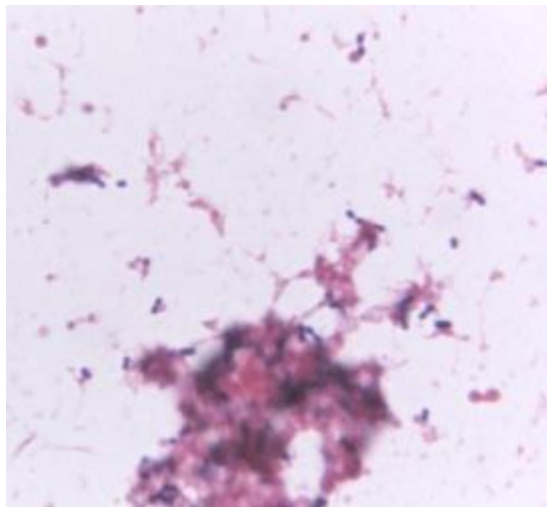
1. Right specimen (more is better)
2. Processing correctly, make a good smear, cover entire slide with thick and thin areas or using cytocentrifuge
3. Fixing (methanol) and staining technique
4. Correct interpretation, based on body site, patient age, patient history
  - Anticipate probable results!

A CLIA high complexity test!

# Ugly smears make ugly Gram stains



Identical blood culture bottle heat fixed (left) and methanol fixed (right)



# Gram stains from swabs

- Standard swabs absorb about 100 microliters and hold 95% of what they absorb
  - Vortex swab in .5 ml sterile saline before Gram stain and plating
- Use new flocked swab collection kits available from multiple manufacturers
- Gram stains suffer from inconsistency compared to other techniques
  - Specimen variables!



# Teach nurses not to use swabs

Mini tip

Regular tip

Syringe

Cup



1x  
0.015 ml

10x  
0.15 ml

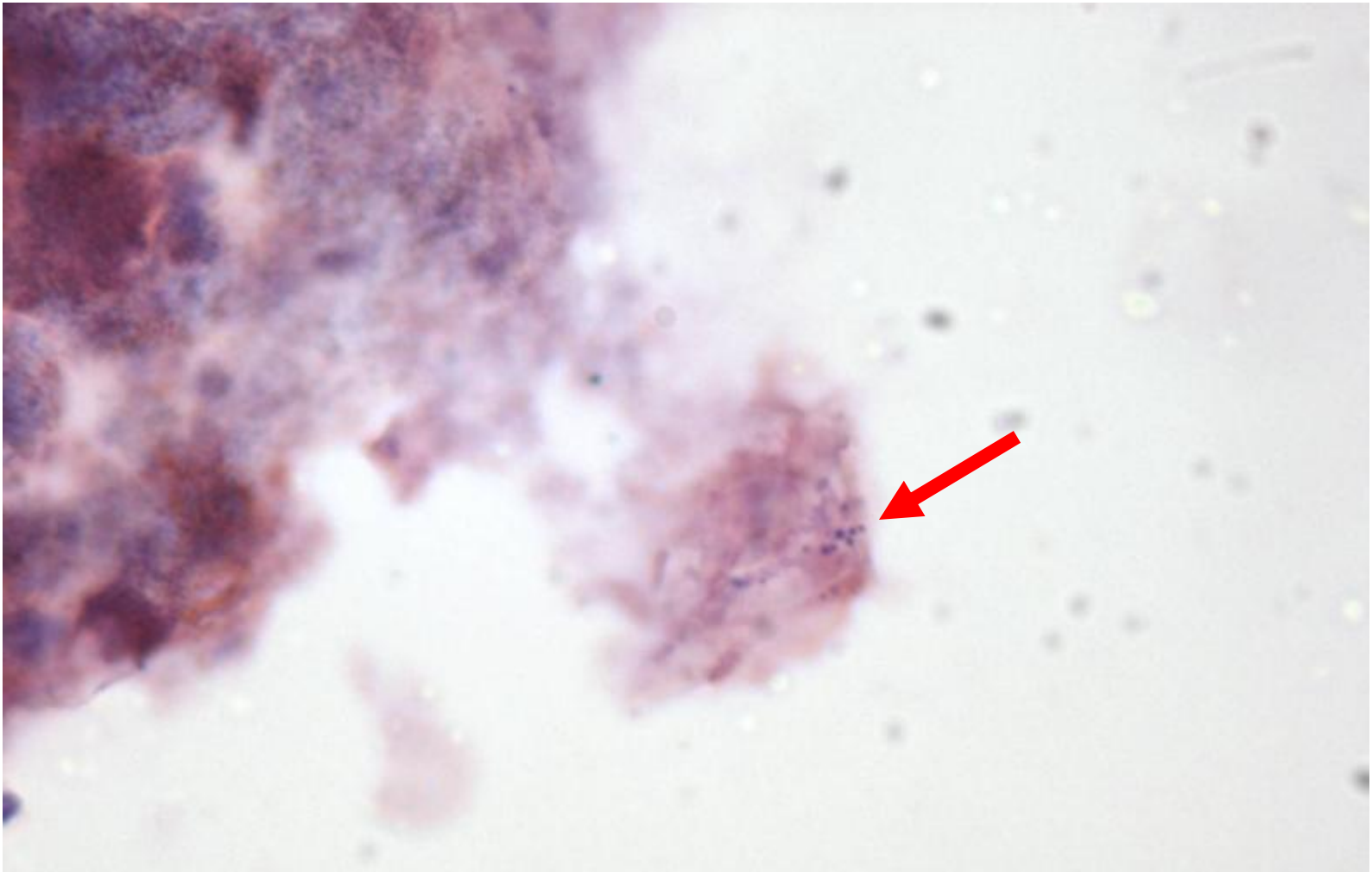
100x  
1.5 ml

1000x  
15 ml

# Gram Stain Case 1

- 19 year old male
- Left knee swab, post-surgical revision
- STAT gram stain, second shift, lab staffed with generalists
- The classic 430 pm Friday scenario

# Case 1, knee swab, what is it?



# Case 1: and the answer is?

1. Amorphous material
2. *Staphylococcus aureus*
3. *Staphylococcus epidermidis*
4. *Corynebacterium* species

# Case 1: the rest of the story

- Classic Monday morning 800 am scenario:
  - Doctor wants to know why the culture did not grow

## Real time patient impact?

- Correlation between gram stain and culture results?
- Lab should have a good gram stain review policy
- Track error rates and track improvements
- Labs should equip 2<sup>nd</sup> and 3<sup>rd</sup> shift generalists for Gram stain success!

# The even bigger rest of the story

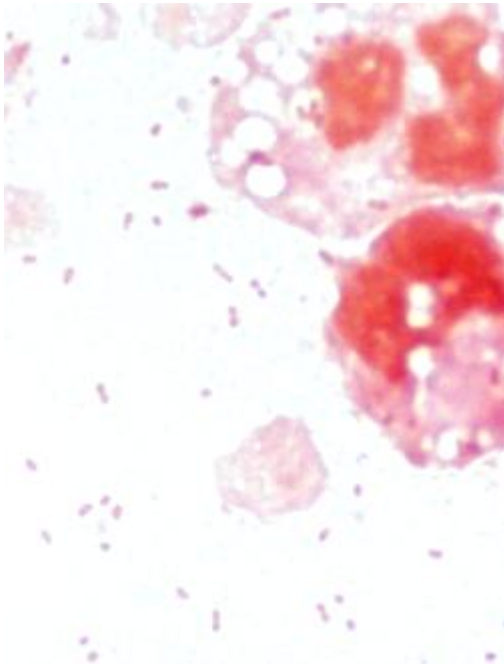
- This case and another spurred a gram stain competency program for generalists and second and third shift techs in a multi-hospital system
  - Lab proficiency is not the same as individual competency
  - Lots of push back, resentment, apathy
  - Must be supported by administration

# It should all make sense

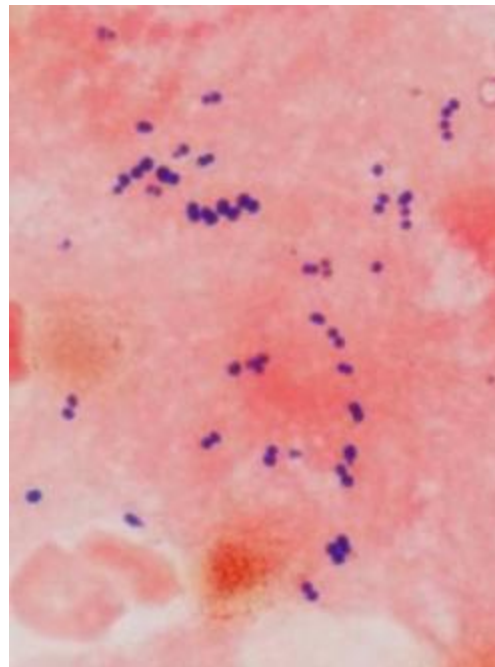
- The Gram stain correlates with the culture
- The culture correlates with the susceptibility
- All three (Gram, culture, susceptibility) correlate with the patient condition
- Most common technical errors: interpreting “junk” as GPC and overlooking gram neg organisms, especially *Haemophilus*

# Reasons for no correlation:

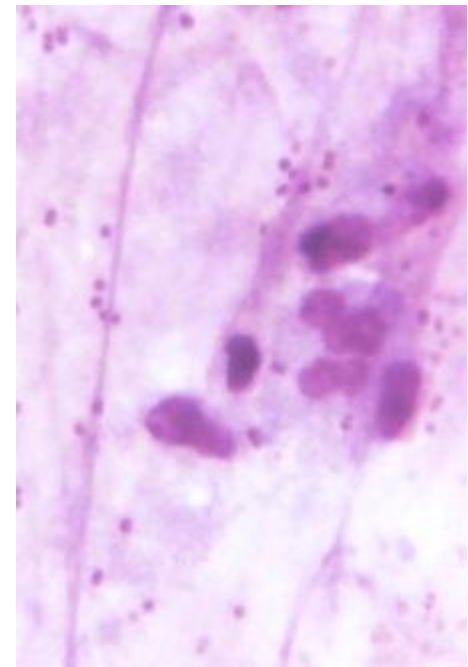
- Wrong slide? – clerical or technical error
- Staining technique
- Less sensitive than culture
- Nonviable, fastidious, or anaerobic organisms



Gram stain looks like  
*Haemophilus* and grew it



Wound culture was no growth  
Despite GPC seen in Gram stain.



Under-decolorized *M. catarrhalis*  
reported as Staph

# Develop a practical QA policy

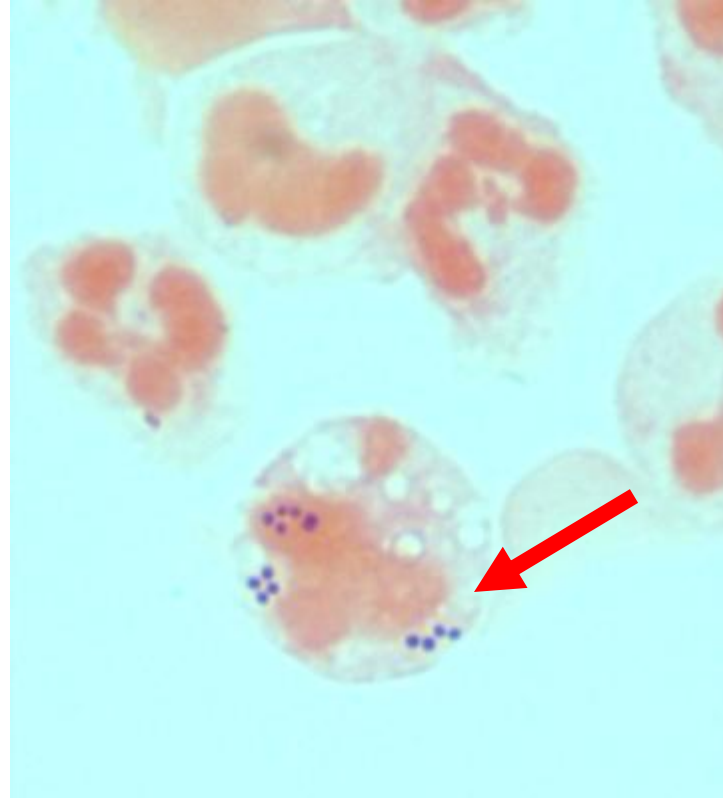
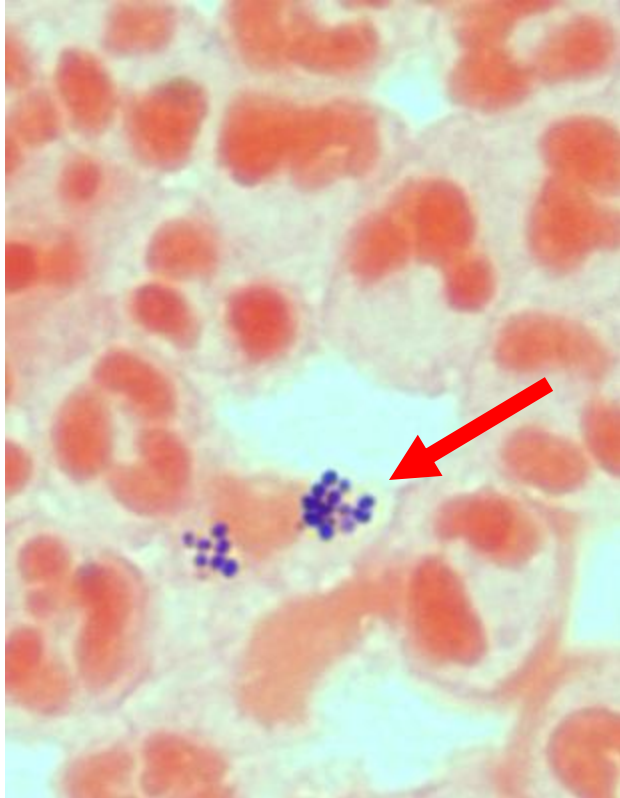
- Organism not seen in Gram but heavy growth on culture
  - lack of sensitivity. In a hurry, wrong slide, poor stain, too thin?
- Organism seen in Gram, but no growth in culture
  - lack of specificity. Anaerobe, dead, fastidious, wrong slide?

Keep stained slide and unstained duplicate for 7 days, at the microscope bench for potential review

## Case 2

- Thirty six year old male
- Swollen left knee, synovial fluid, aspirated, submitted in 50 ml syringe
- Grossly purulent
- Specimen is cytocentrifuged – makes a beautiful easy-to-read stain!

# Case 2, synovial fluid, what is it?



# Case 2: and the answer is?

1. *Staphylococcus aureus*
2. Coagulase-negative *Staphylococcus*
3. *Neisseria gonorrhoeae*
4. *Kingella kingae*

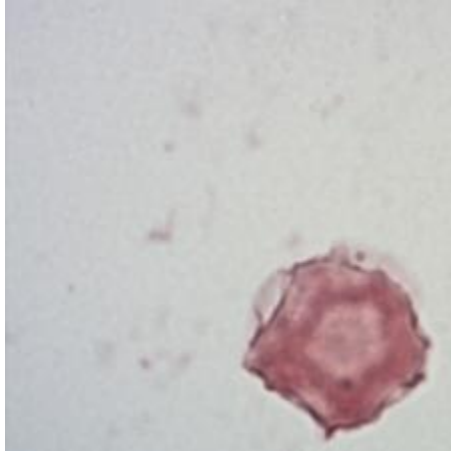
Real time patient impact?

# Case 3

- 23 yr old female, corneal ulcer, contact lens wearer, corneal scraping, special ringed slide
- Ophthalmologist gave lab a “heads up” on suspected pathogens
- Only three of the following structures seen on entire slide – scanned on low power first



# Case 3 cornea scraping, what is it?



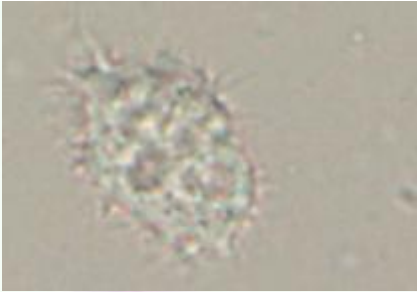
## Case 3: and the answer is?

1. *Cryptosporidium* oocyst
2. *Acanthamoeba* cyst
3. Microsporidia spore
4. Pollen grain

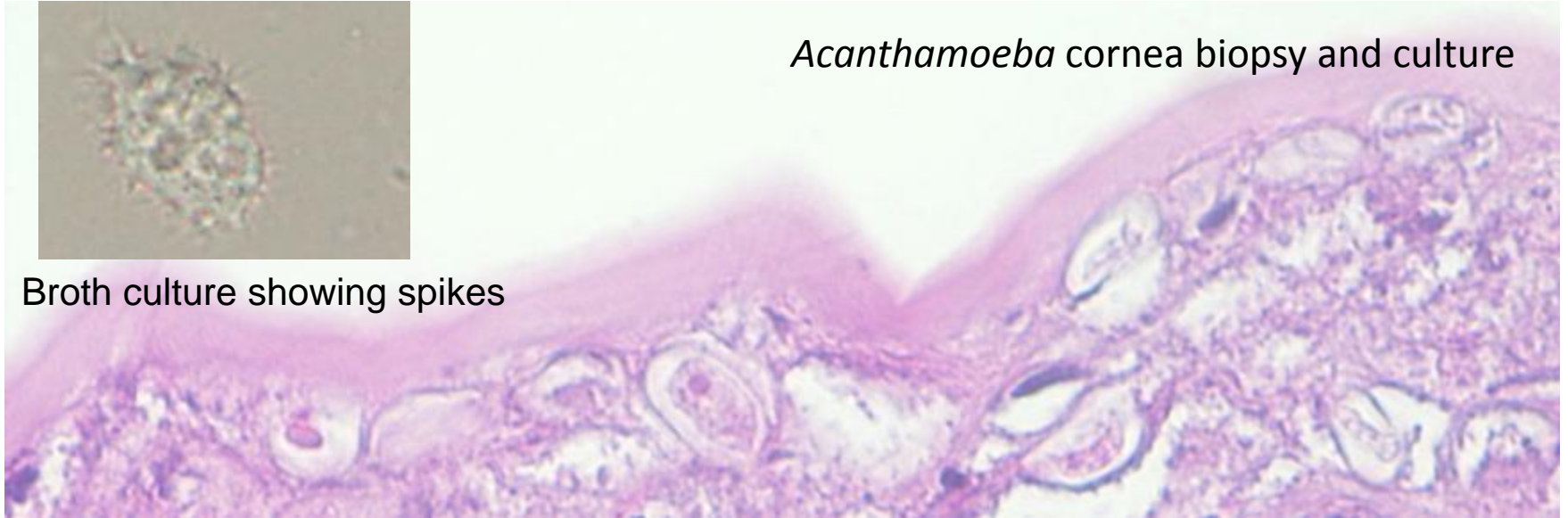
# Case 3: the rest of the story

- Patient used “home made” contact lens solution with tap water and table salt
- Real time patient impact?

*Acanthamoeba* cornea biopsy and culture



Broth culture showing spikes



*E. coli* overlay on non-nutrient agar

## Case 4

- 51 yr old male from Mexico, 18 month history of progressive vision difficulty and headache
- admitted for cranial resection of mass at base of skull, posterior fossa, 6/28/2011
- Pathology report: consistent with schwannoma

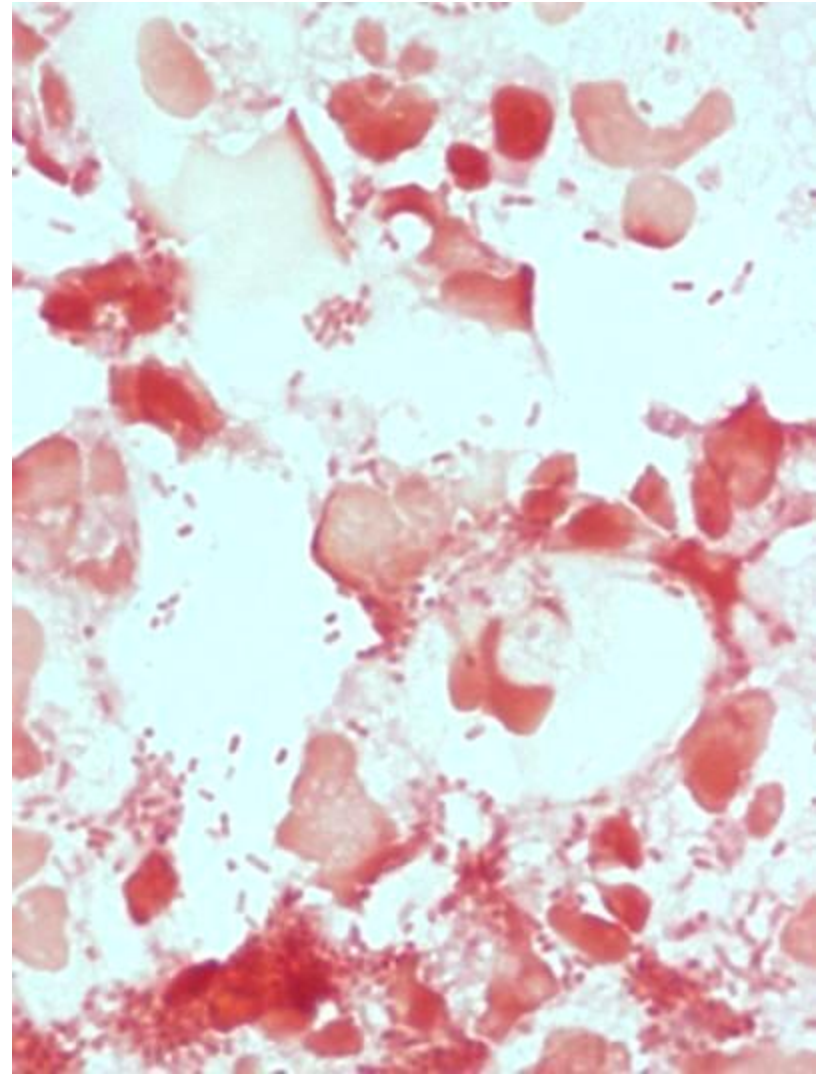
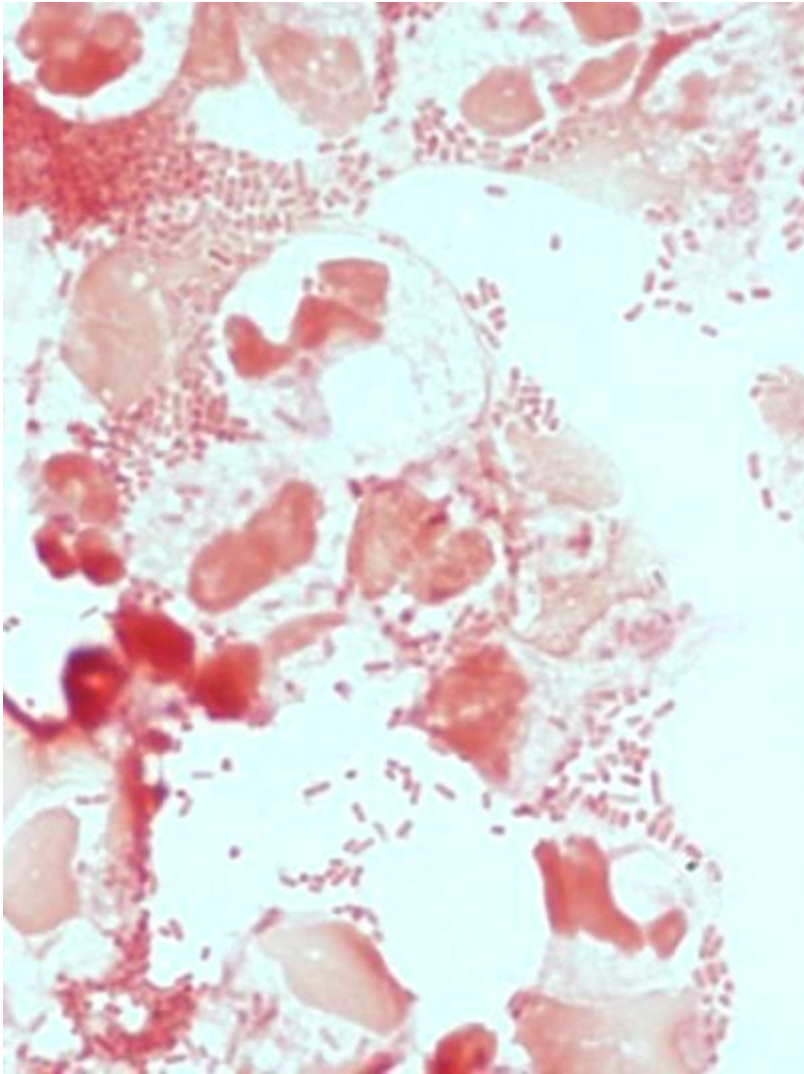
# Case 4

- Placement of right occipital ventriculostomy
- Post-op patient has **HSV infection** on face/eye
- Persistent leukocytosis despite steroid tapering, Peripheral WBC 18.3
- Afebrile
- Pus noted at site of drain, CSF collected from drain, drain removed

# Case 4 CSF values

- Glucose: 1
- Protein: 82
- Cell count: colorless, hazy, 163 RBC, 398 WBC, 100% neutrophils
- Gram stain: Many WBCS, Many GNR

# Case 4



Cytocentrifuged CSF Gram stain 1000x oil immersion

# Case 4: and the answer is?

1. *Enterobacter aerogenes*
2. *Haemophilus influenzae*
3. *Pseudomonas aeruginosa*
4. *Moraxella catarrhalis*

Real time patient impact?

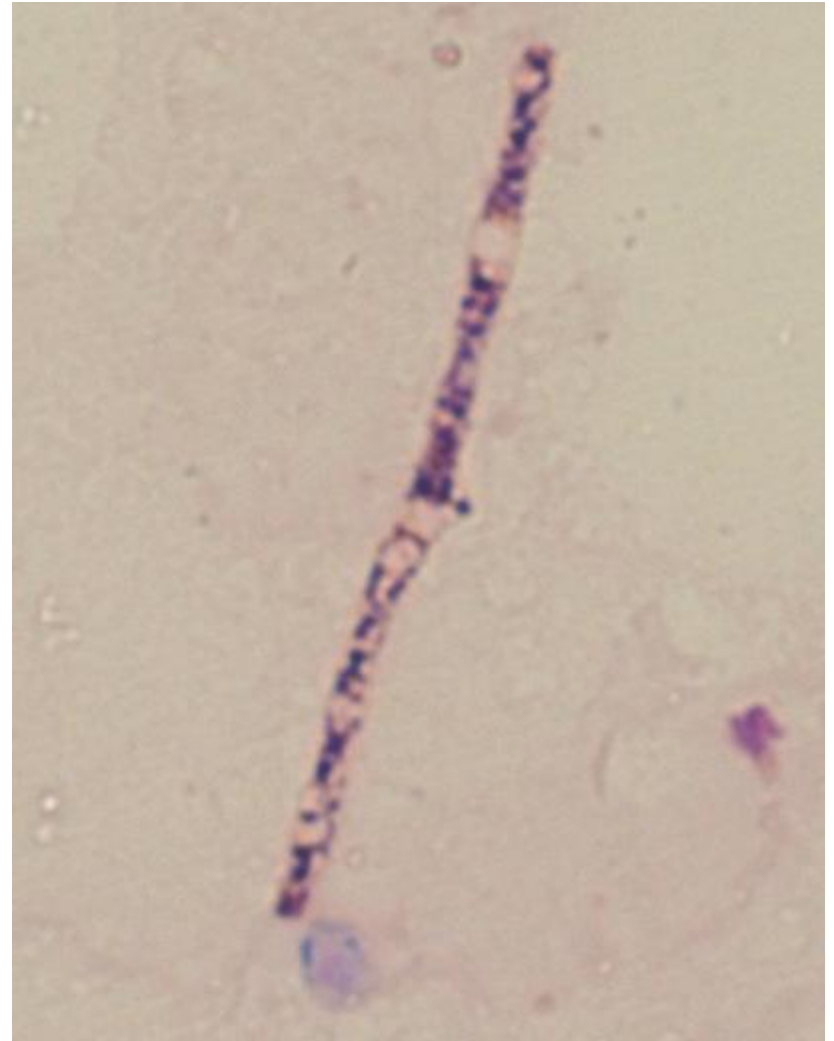
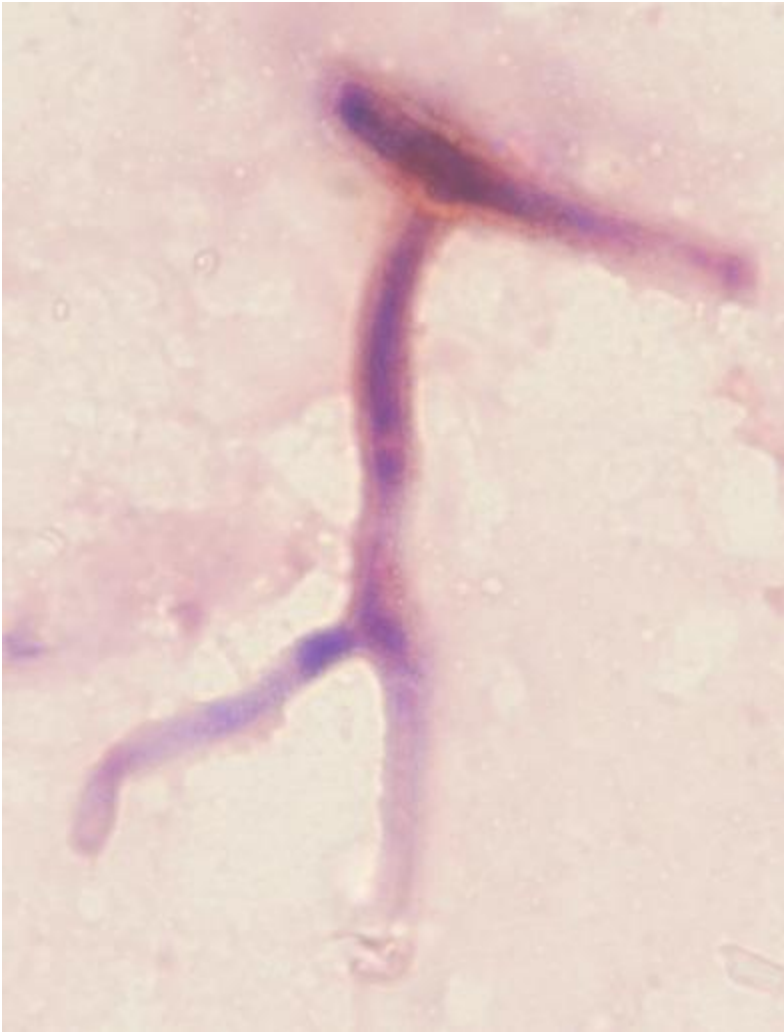
# Nosocomial \_\_\_\_\_ Meningitis Risk Factors

- Neurosurgery
- CSF drainage device
- Isolation of \_\_\_\_\_ from other body site
- Prolonged antimicrobial therapy inactive against \_\_\_\_\_
- VP shunt and drain patients have different range of CSF pathogens
- REFERENCE: Stephen Parodi, Arno Lechner, Regina Osih, Paul Vespa, and David Pegues Nosocomial Enterobacter Meningitis: Risk Factors, Management, and Treatment Outcomes, Clin Infect Dis. (2003) 37(2): 159-166

# Case 5

- Three yr old female, ALL, chemotherapy
- Rt knee lesion, one week ago, now three new 0.5 cm lesions, rt forearm, rt leg
- Biopsy from 1.0 cm lesion on rt knee, submitted for histology, routine, fungal, AFB cultures
- Original STAT gram stain reported as NOS, pedi ID physician requests review

# Case 5, knee tissue, what is it?

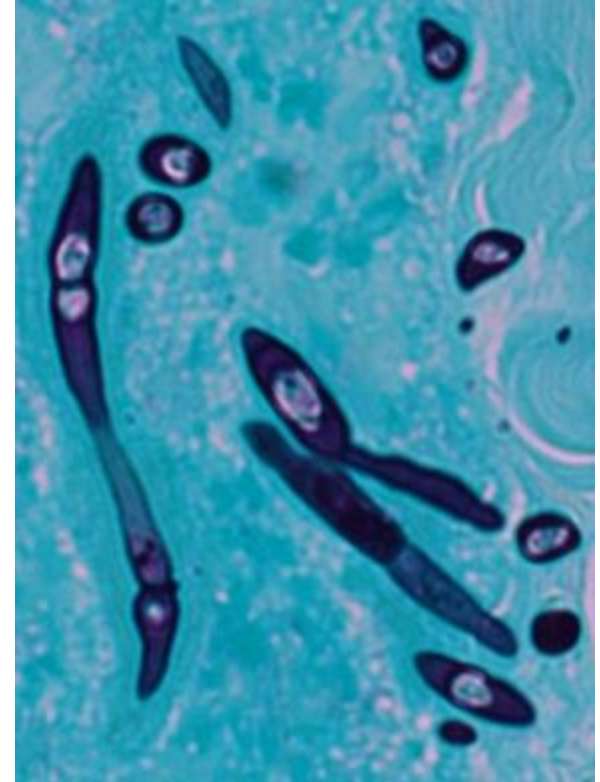


# Case 5: and the answer is?

1. Aseptate fungal hyphae
2. Septate fungal hyphae
3. Yeast pseudohyphae
4. Artifact

**Real time patient impact?**

Gram is faster than histology or journey to fungus lab

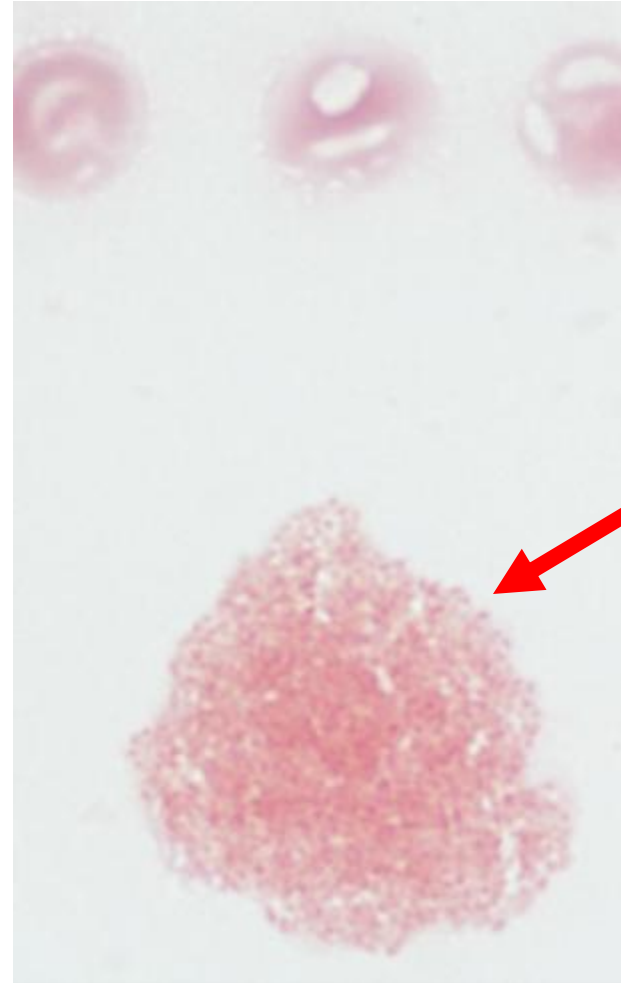


GMS stain of same specimen

# Case 6

- 51 year old female, Mexican national
- Intermittent fevers of several weeks duration
- 30 pound weight loss during this time
- No insurance
- Blood cultures drawn in ED, patient went home
- Blood cultures x 2 positive at 5 days incubation

# Case 6 blood culture, what is it?



# Case 6, and the answer is?

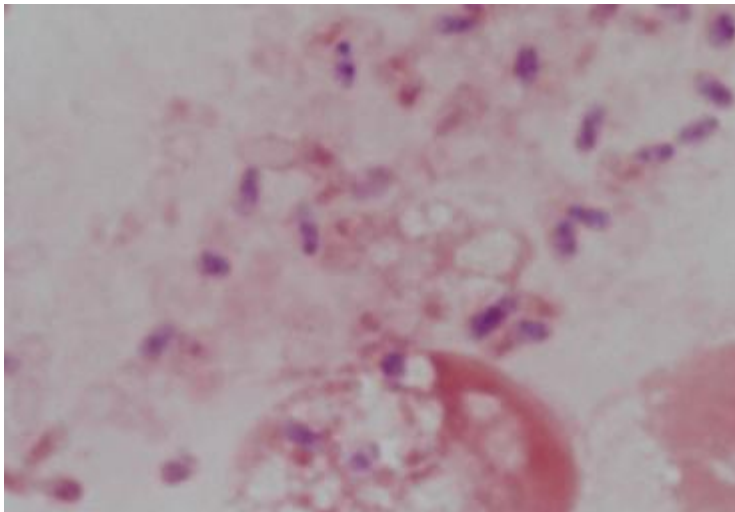
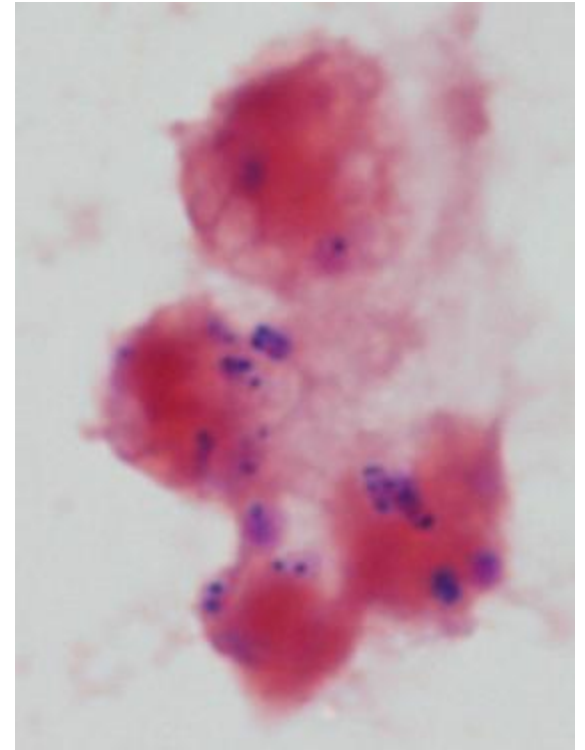
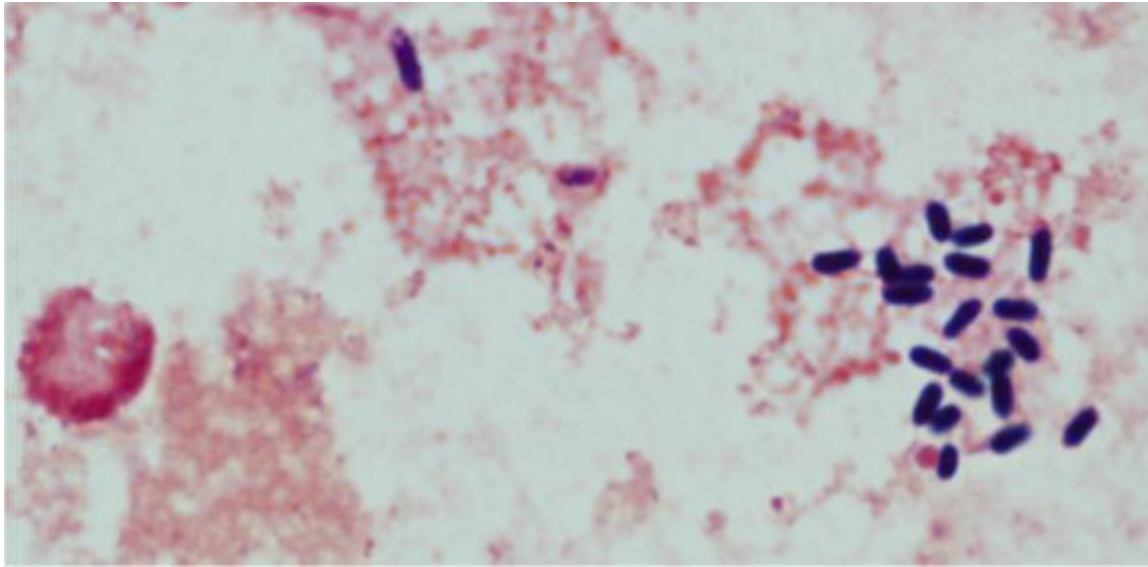
1. *Haemophilus influenzae*
2. *Campylobacter fetus*
3. *Bartonella* species
4. *Brucella* species

Real time patient impact?

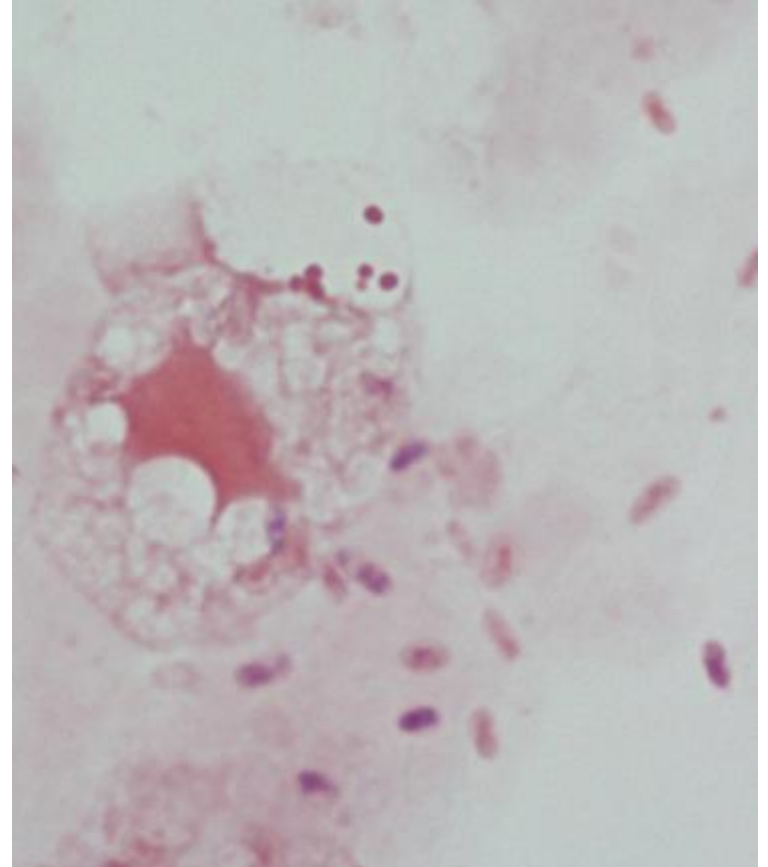
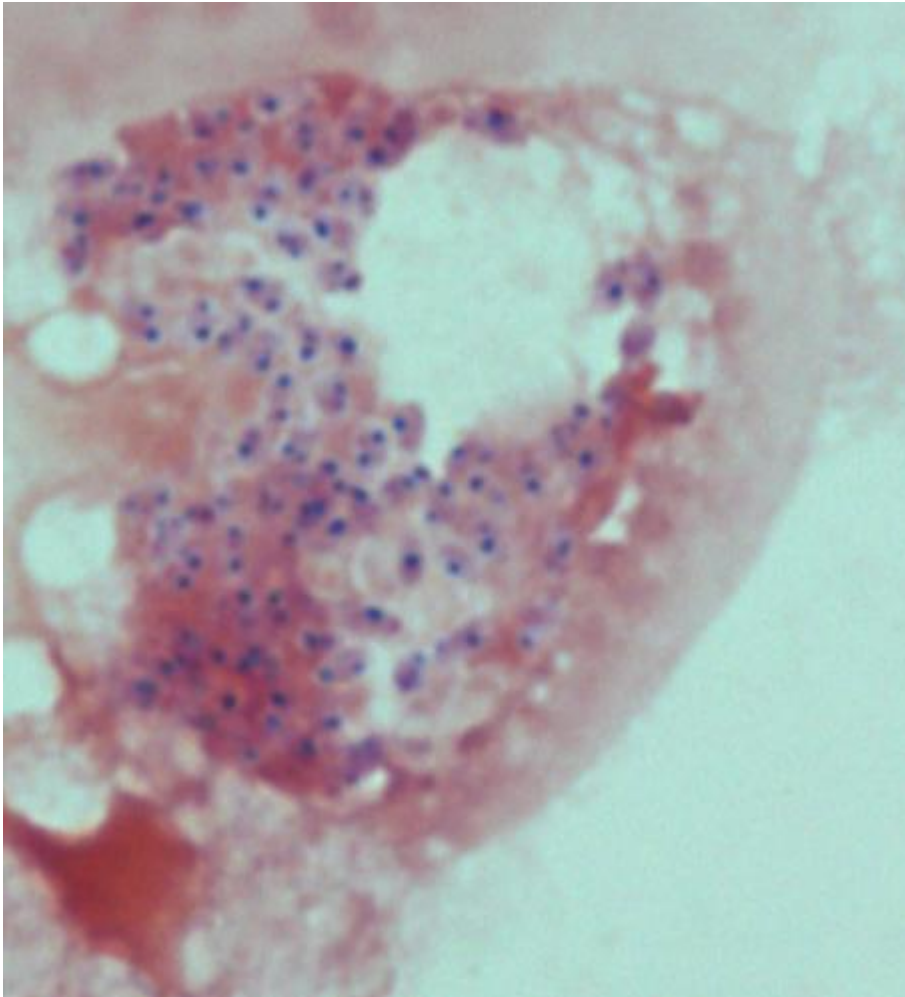
# Case 7

- 56 year old male lung transplant patient, cattle rancher
- FUO three months
- Kidney failure
- Physician asks for urine Gram stain
- Urine culture is no growth

# Case 7, urine, what is it?



# Case 7

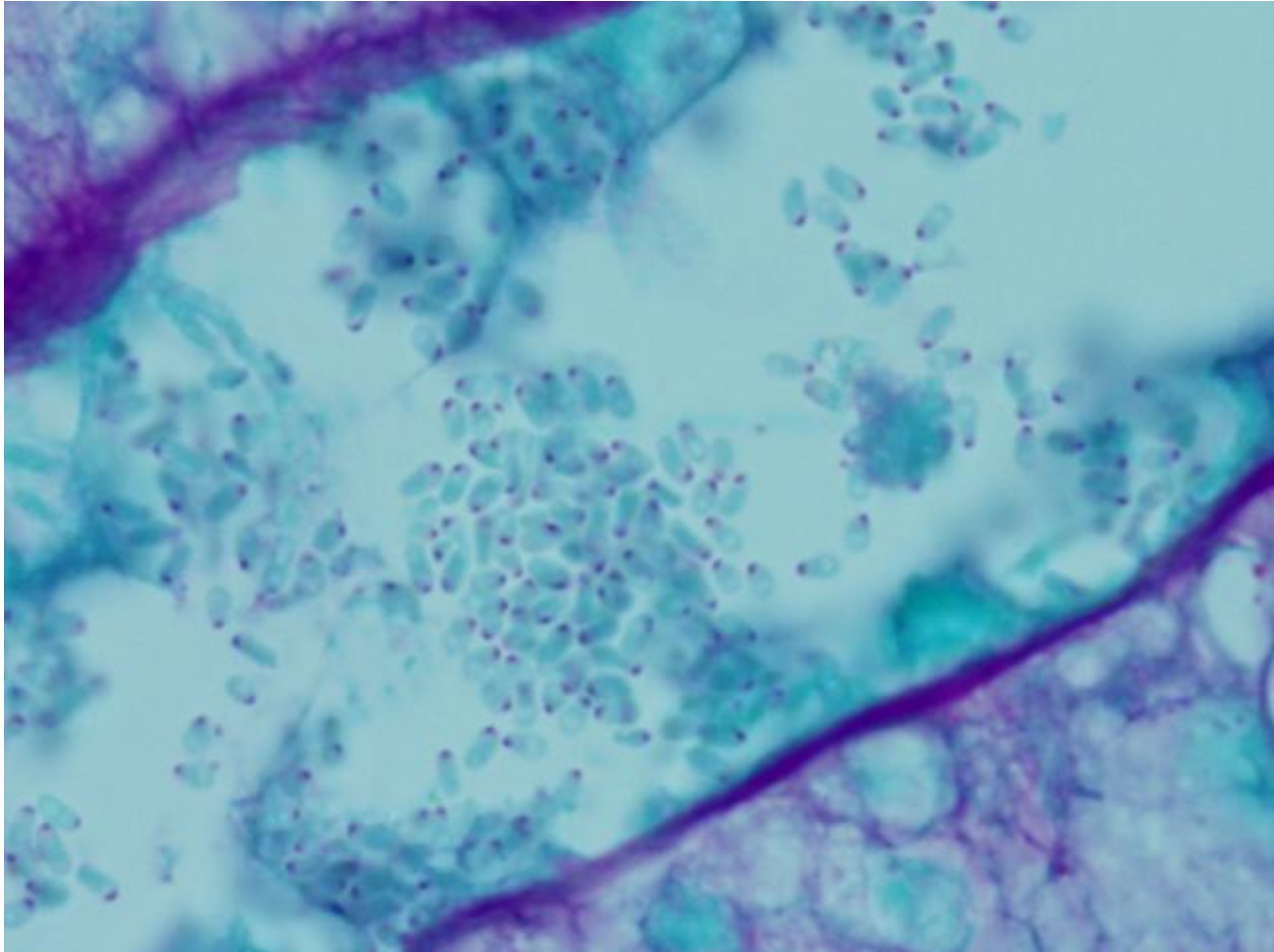


# Case 7: and the answer is?

1. Microsporidia
2. Anaerobic gram pos rods
3. *Propionibacterium* species
4. Yeast
5. *Corynebacterium urealyticum*

Real time patient impact?

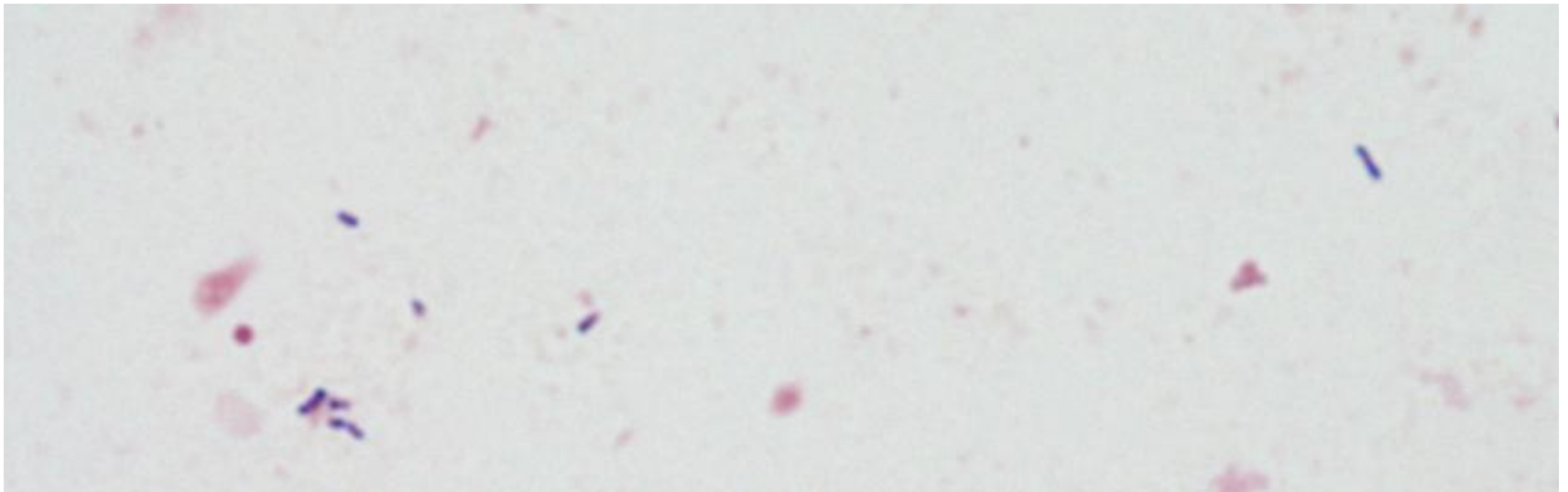
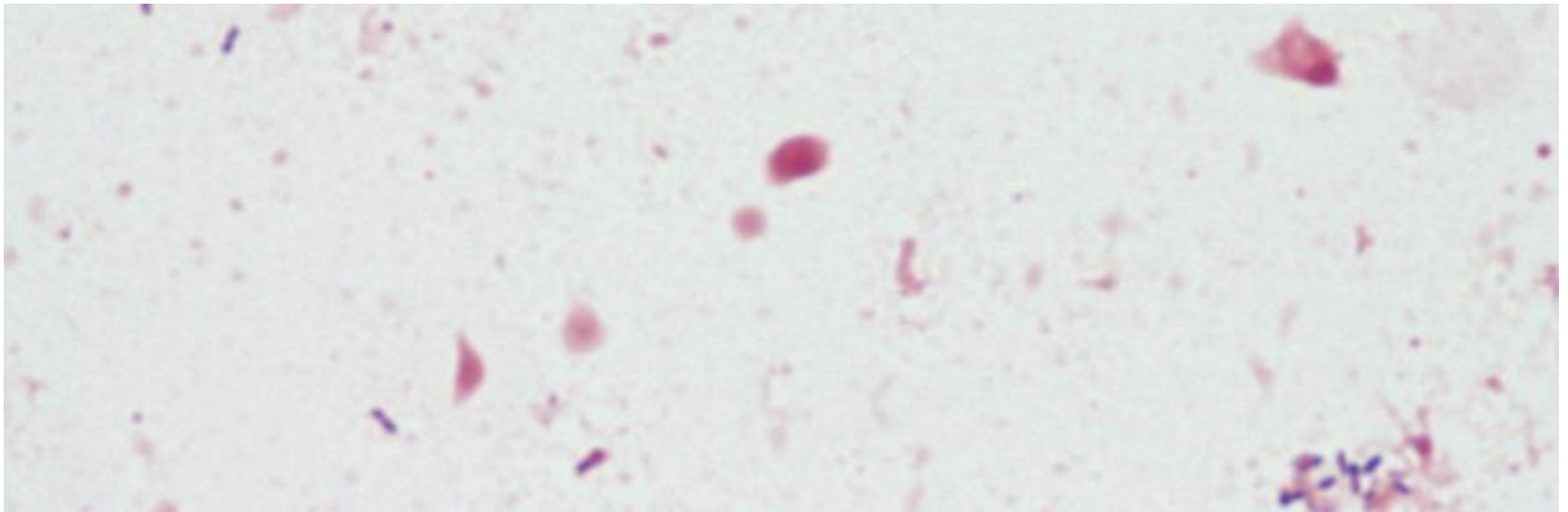
Case 7, same organisms were seen by pathologist  
in kidney biopsy, PAS stain



# Case 8

- Forty seven year old male
- Resection of left temporal arteriovenous malformation (AVM).
- Two weeks after surgery worsening aphasia and headaches
- Brain abscess: craniotomy, purulent debris, debridement and irrigation

# Case 8



# Case 8: and the answer is?

1. *Propionibacterium*
2. *Actinomyces*
3. *Streptococcus pneumoniae*
4. *Clostridium*
5. Under-decolorized *Fusobacterium*

Real time patient impact?

# Conclusion

- Gram stain is a “real-time multiplex” test that is still quite useful even in the molecular era
- Still one of the best tests we have, detects/differentiates bacteria, fungi, parasites
- QA and review can improve processes
- Improvements such as flocced swabs, limiting use of swabs, cytocentrifugation and methanol-fixation can improve quality
- May have immediate real-time patient impact, guiding diagnosis and therapy



Texas: where everything is bigger, even the bacteria!