Key 2: Making a beautiful slide

Making a beautiful slide

- Minimum info on the slide:
  - Name
  - Accn number
  - Body site
  - Use stain resistant computer labels

For some of you, an instrument is making the slides!

- **Always make two! Always Always!!!!!!!!!!!!**
- **Slide retention policy: keep stained and unstained for at least a week**
- Very specimen-dependent, we will discuss some tips for:
  - Sputum
  - Tissues
  - Very small volume specimens
  - Surgical specimens
  - New, inexperienced techs

Making a beautiful slide

- If it looks bad before you stain, it will probably look bad under the scope.
- If you can’t see it on the slide before you stain it, you won’t be able to see anything under the scope.
- Avoid glass etchers
- Avoid wax pencils

Some real life examples

Don’t use the cheapest slides like they do in UA. Some recommend alcohol cleaning slides before use.

Applying the specimen to the slide

- Roll swabs gently and then make some dots!
- Sputum: use a pipette or swab to get the pus stuff, this is where the PMNs and bugs are!
  - Remember that real sputum is pus from the lungs!
  - Stay away from the clear stuff

What about automation?
See supplement for "robo-grammers"

Tissue touch preps and the gram stain sandwich!

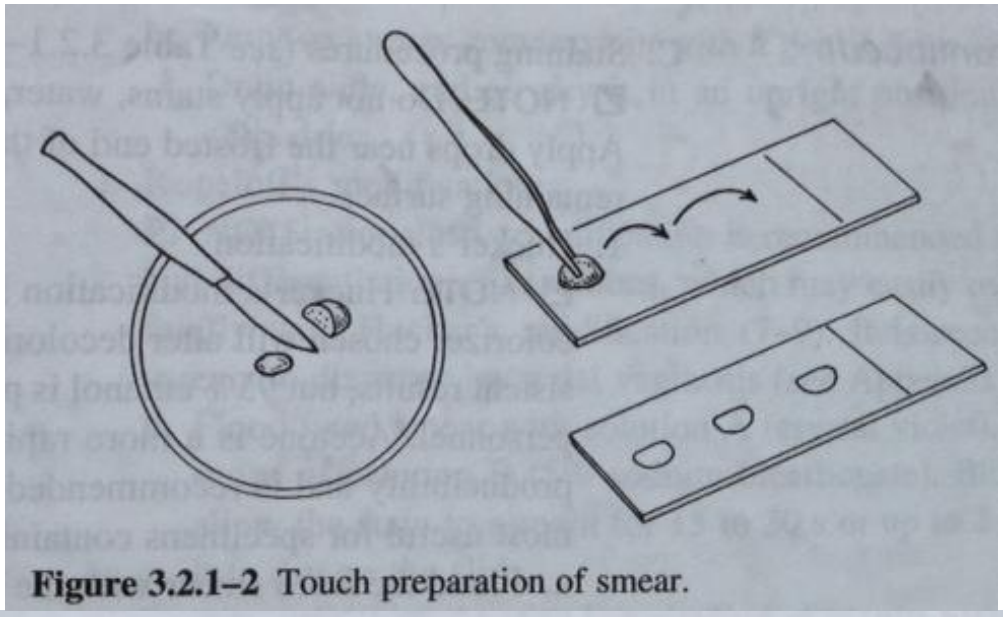


Figure 3.2.1–2 Touch preparation of smear.

Images from CMPH
3rd ed volume 1, 3.2.1.5 and 3.2.1.6

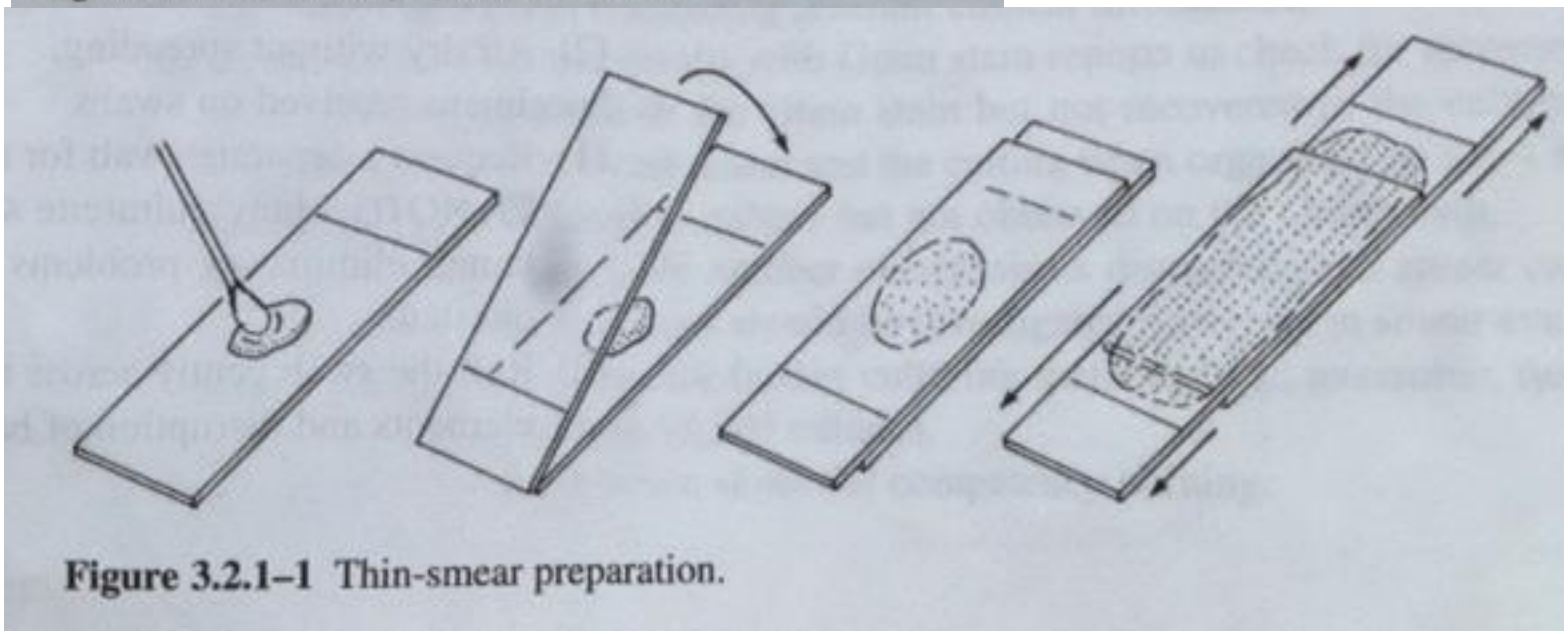


Figure 3.2.1–1 Thin-smear preparation.

The sweet 16 ways to mess up a Gram stain

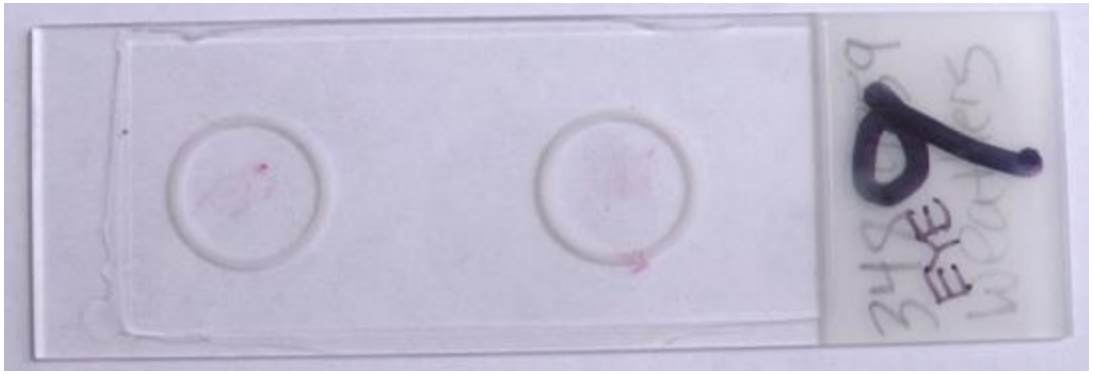
1. “Did not stick to the slide” false negative, synovial fluid
2. “Too thin to see” false negative
3. “Looked at the wrong area of the slide” false negative
4. “Overcooked-lyse all the WBCs and bacteria” heat-fixation false negative
5. “Motile bacteria” false negative
6. “Big splash” false positive
7. “Glove flora” false positive
8. “Bibulous/blotting paper tattoo” false positive

Sweet 16 continued

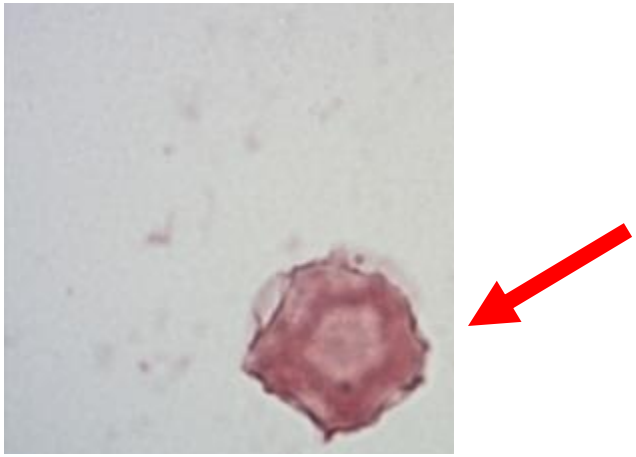
9. “Crystal violet precipitate looked just like a gram positive cocci” false positive - the most common error among inexperienced techs.
10. “Wax pencil debris looked just like a bacteria” false positive
11. Looked at the wrong slide clerical error
12. “Too thick to see anything”
13. “Pressure washer rinse” false negative
14. Overdecolorized
15. Underdecolorized
16. Variably under and overdecolorized on same slide

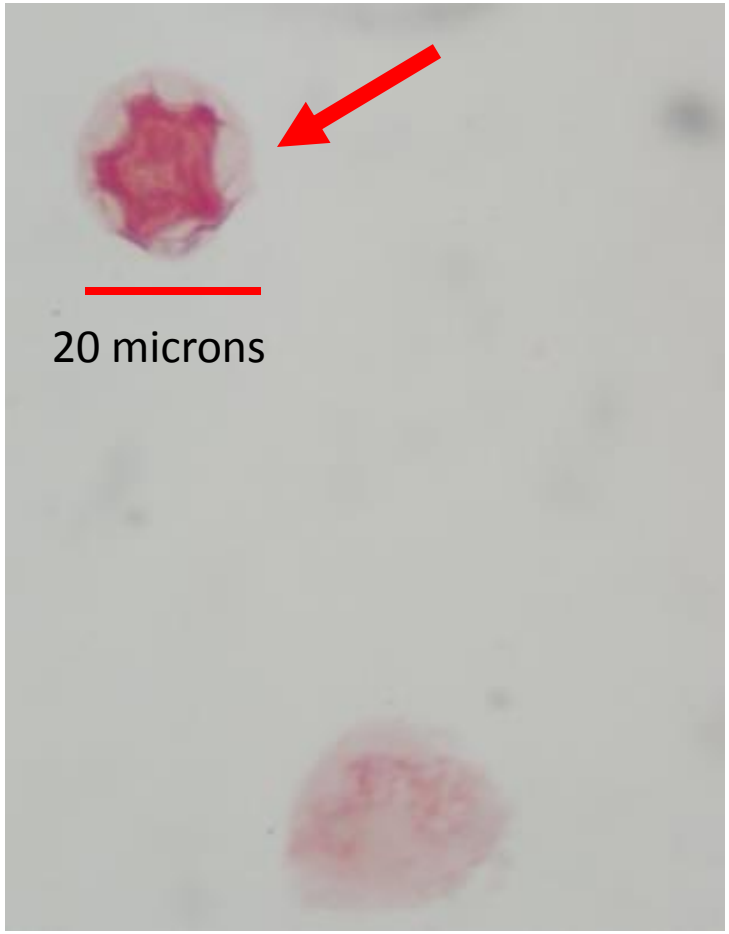
Frosted ring slide case

- 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.



Cornea scraping, what is it?



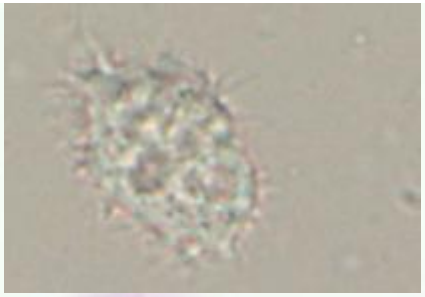


And the answer is?

A. *Cryptosporidium* oocyst
B. *Acanthamoeba* cyst
C. Microsporidia spore
D. Pollen grain

The rest of the story

- According to conversation with the doctor: “Patient used home made contact lens solution with tap water and table salt.”
- Real time patient impact?
- What did the doctor say?_____



Broth culture showing spikes

Acanthamoeba cornea biopsy and culture

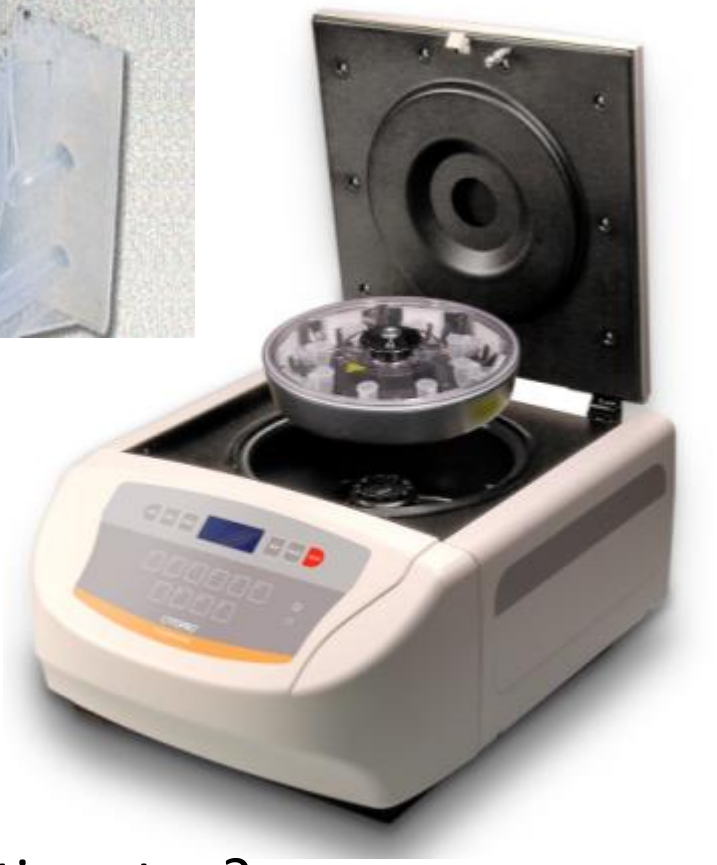
E. coli overlay on non-nutrient agar

Too many hands on the slide?

- In bigger labs, one person might make the slide, another stains it, and another reads it.
- Make the “making part” part of competency for lab assistants.
- Refuse to read crappy slides but show how you want them made.
- Keep unstained and stained example slides near set up area.

Cytocentrifuge if you can

- CSF
- Synovial fluid
- BAL?
- Flocked swabs?
- Urine?

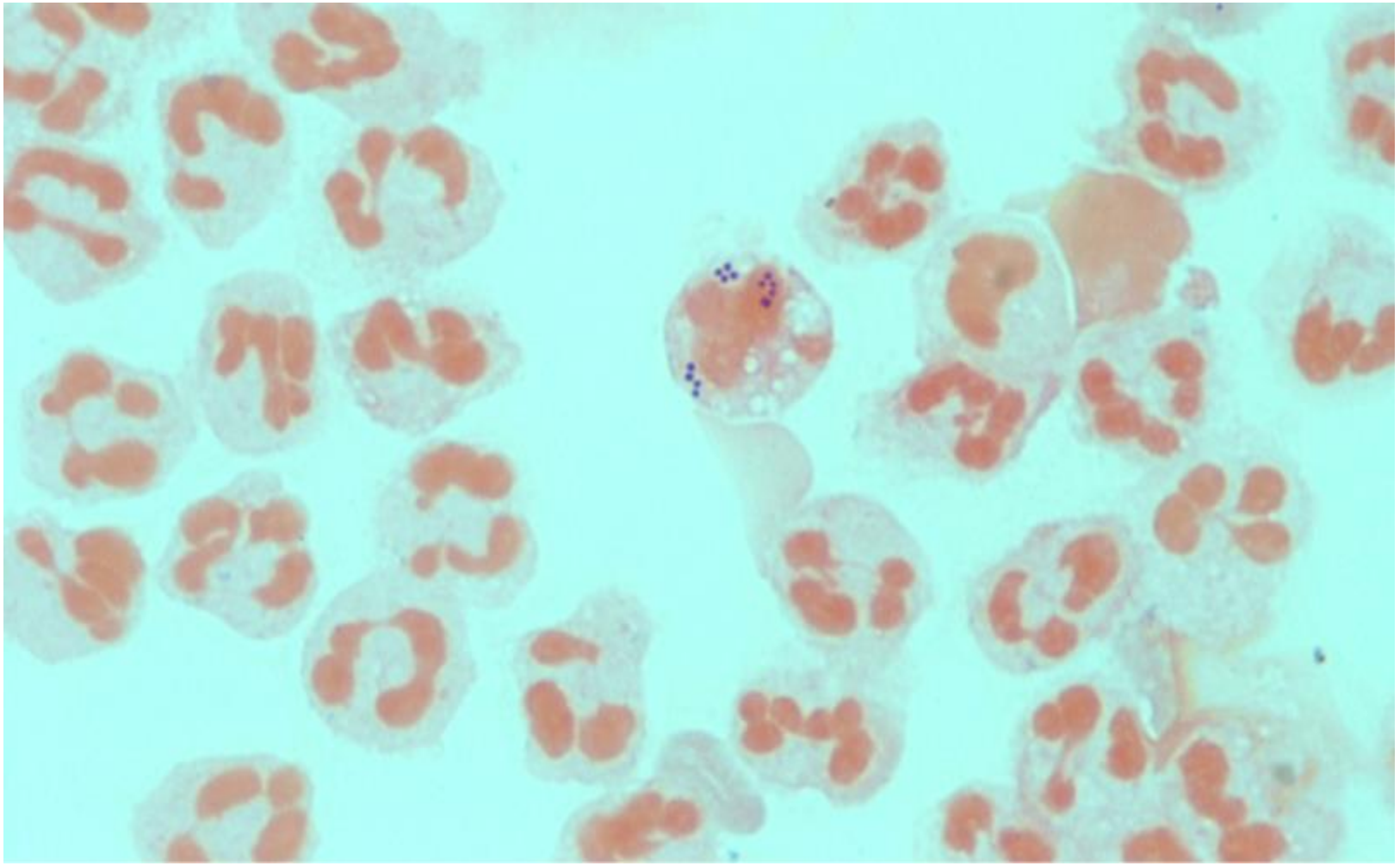


Reality: Can you bill for the extra labor and cost of the cytocentrifugation step?

Cytocentrifuge case

- Thirty six year old male
- Swollen left knee, synovial fluid, aspirated, submitted in 50 ml syringe
- Grossly purulent
- Reported as Many PMNs but NOS, physician wants review
- Specimen is cytocentrifuged – makes a beautiful easy-to-read stain!

Cytocentrifuged synovial fluid, what is it?



And the answer is?

A. *Staphylococcus aureus*
B. Coagulase-negative *Staphylococcus*
C. *Neisseria gonorrhoeae*
D. *Kingella kingae*

- Real time patient impact?
- Cytocentrifuge increases detection sensitivity from 100,000 to 10,000 organisms/ml!

Cytocentrifuge

- How much specimen?
- How fast and how long to centrifuge?
- I prefer the dual ring version, make one thick and one thin
- Pre-dilute viscous specimens in a test tube
  - Saline, formalin, albumin as diluent?

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Concentrated Gram Stain Smears Prepared with a Cytospin Centrifuge

CAROL J. SHANHOLTZER,[1]* PAMELA J. SCHAPER,[1] AND LANCE R. PETERSON[1,2]

Microbiology Section, Laboratory Service,[1] and Infectious Disease Section, Medical Service,[2] Veterans Administration Medical Center, Minneapolis, Minnesota 55417

Specimen	Sample vol (drops)	Centrifugation[a] time (min)
High viscosity (e.g., bile, purulent material, synovial fluid)	1	5
Moderate viscosity (e.g., pleural fluid, peritoneal fluid)	5	5
Low viscosity (e.g., CSF, peritoneal dialysate)	10[b]	10

[a] At 2,000 rpm.
[b] 0.5 ml.

Key 2 Summary

- Use the whole slide
  - Make thick and thin areas
- Make two slides, stain one, keep both
  - Slide retention policy
- Use the cytocentrifuge when indicated
  - Synovial, CSF, other normally sterile fluids
- For sputum, get that nasty pus
  - not the clear stuff
- Use frosted ring slides
  - for cornea and small specimens.

Random Gram stain challenge, sputum