The Gram Stain:
Five Keys to Lock in Great Gram Stains
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The starfish story

• Three yr old female on chemo for acute lymphocytic leukemia
• Rt knee lesion, one week ago, now three new 0.5 cm lesions, rt forearm, rt leg
• Biopsy (not swab) from 1.0 cm lesion on rt knee, submitted for histology, routine, fungal, AFB cultures
• Original STAT gram stain reported as “no organisms seen”
• Pedi ID physician requests review
Importance of doc talk

• ID doc specifically called me and said:
  – “This little girl is really sick. She has these progressive lesions turning from red to black, I don’t know what they are. Can you review the slide for me? She is neutropenic, so it could be anything.”

If you make enough differences, you make a sameness!
Knee tissue, what is it?
And the answer is?

A. Aseptate fungal hyphae
B. Septate fungal hyphae
C. Yeast pseudohyphae
D. Artifact

Real time patient impact?
Gram is faster than histology or journey to fungus lab, turning “unknowns into knowns”
And after getting the results?

• The doctor said _______________!
The gram stain is still the best, fastest and cheapest STAT multiplex 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!
This is your reality, right?

• Micro is slow: doctors are used to it and so are we! Are we accepting mediocrity?
• A lot of the specimens we get are crappy and nobody seems to care
• It takes days, weeks, months to train a chemist but years to train a microbiologist!

So what are we gonna do about it?
Objectives

• Improve clinical specimen Gram stain quality and positively impact patient care by applying **five keys in a real patient case-based approach**:
  1. Appropriate and enough specimen
  2. Making a beautiful slide
  3. Staining it right
  4. Correctly interpreting the results
  5. Reporting the results in an understandable way

• Incorporate all five gram stain keys into competency assessment.

• Implement a reasonable QA plan in context of new CAP checklist requirements
Basic review

• Gram stain detects, differentiates microorganisms
• Multiplex, real time: detects bacteria, fungi, some parasites, right now!
• Limitations: detects cell walls
• Insensitive compared to culture, but faster!
• Gram stain has four time-dependent reagents:
  – crystal violet
  – Gram’s iodine
  – Decolorizer acetone/alcohol, timing is critical
  – safranin
• Gram neg is red, Gram pos is blue/purple
Basic morphologies
Doug Bug Drug Triangle: Detect, identify, eliminate.

Specimen

Doug

You are here.

Gram stain, 20 minutes

Rapid flu test, 20 minutes

Culture, 24 hours

Drug

Antibiotics susceptibility 6 to 24 more hours

Doug Bug Drug Triangle: Detect, identify, eliminate.
Q:
The Gram stain will not detect:
A: Microsporidia, Acanthamoeba
B: Chlamydia, Mycoplasma
C: Mycobacteria, Nocardia
Q:

The limit of detection for Gram stains is ________ organisms per ml of fluid or tissue:

A. $10^3$
B. $10^4$
C. $10^5$
D. $10^6$

References:
Gram stain clinical impact

• Since it is the first step, it is also our first chance to help, or to mess up!

• CLIA high complexity test

• FACT: THE GRAM STAIN IS A NON-STANDARDIZED SUBJECTIVE TEST PERFORMED ON NON-STANDARDIZED SPECIMENS

• If patient gets empiric antibiotics, does it matter?
  – Sterile body site results have most clinical impact

• “No organisms seen” can actually be helpful.

• Helps diagnose infections and guide therapy real-time
  GNR versus GPC versus Yeast versus Fungi
Q:

• What is the sensitivity and specificity of specimen gram stain result correlated to culture growth results?

A. It depends on the body site
B. These statistics cannot be calculated
C. 95% sensitive, 90% specific
D. 67% sensitive, 95% specific

Reference:
The “real time” part

• In many labs, ROUTINE gram stains are downplayed.
• Maybe done only once per shift?
• Doctors actually used to NOT getting timely results so they don’t check.
• Try doing them multiple times per shift, including second/third.
• Remember, NOT seeing things is good too.
• With blood cultures, fast TAT = less death!
Gram stain is dependent on five keys:

- The right specimen (more is better)
- Processing specimen correctly, making a good smear, covering the entire slide with thick and thin areas or using cytocentrifuge
- Good fixing and staining technique
- Correct interpretation, based on body site, patient age, patient history
- Clear reporting
  - Anticipate probable results!
Key 1: appropriate specimen
Key 1: appropriate specimen

- Prefer BAL to sputum
- Prefer specimen to swab of specimen
- Prefer E-swab to regular swab
- Prefer big swab to small one
- Prefer dual swab to single one
- Prefer more compared to less
- Prefer and require FULL blood culture bottles
- Verbalize and publish preferences
- Give in-services to nursing staff

REALITY: you will still get a lot of swabs!
Sputum cultures

• Rejection criteria

• The Epi and PMN thing
  – Examine 20-40 fields on low power and average

• Do you use?
  >10 SEC/LPF
  >25 SEC/LPF
My real-time nurse versus sputum conversation

- Father in law with COPD and lung cancer being admitted for possible pneumonia
- Nurse actually says: “Honey we need to get you to spit in this cup so we can figure out what’s causing your cough.”
- I say: “What the @#$%#@!”
Gram stains from swabs

- Standard swabs absorb about ____? microliters and hold ____?% of what they absorb.
  - Vortex swab in .5 ml sterile saline before Gram stain and plating – but now I have another tube!
- Use new flocked swab collection kits available from multiple manufacturers.
- Gram stains suffer from inconsistency compared to other techniques.
  - Specimen variables!
Swab gram stain processing debate

• Sterile slides, yes or no?
• Make the slide first?
  – Advantages, disadvantages?
• Streak the plates first?
  – Advantages, disadvantages?
Gram stain from swab, decubitus

Swabs: only scratching the surface!
Another surface wound swab

Q score to the rescue?
Special note on re-staining swabs

• When you streak swab across media, you are giving the bugs food.

• There will be more bugs on the swab if it was not immediately refrigerated after inoculation, therefore, results may not correlate with original gram stain!
• Gets more specimen
• Releases more specimen
• Makes great cytocentrifuge gram stains
• Can be used for anaerobes
• Use the eluted liquid to inoculate media instead of the swab, gives you more specimen and some standardization.
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
Should we ever gram stain urine or stool?

• May make a difference in some cases
• Like the next case you are about to see
• For stool, if it is really watery, mucoid or bloody, these can be seen:
  – Vibrio
  – Campylobacter
  – Microsporidia
Urine case

• 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
Urine, what is it?
And the answer is?

A. Microsporidia
B. Anaerobic gram pos rods
C. Propionibacterium species
D. Yeast
E. Corynebacterium urealyticum

Real time patient impact?
Same organisms were seen by pathologist in kidney biopsy, PAS stain
Making a beautiful slide

• Minimum info on the slide:
  – Name
  – Accn number
  – Body site
  – Use stain resistant computer labels
• Always make two! Always Always!!!!!!!!!!!!
• Slide retention policy: keep stained and unstained for at least a week
• Very specimen-dependent but 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!
  – See example slides

• 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
Tissue touch preps and the gram stain sandwich!

Images from CMPH 3rd ed volume 1, 3.2.1.5 and 3.2.1.6
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
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
• These are great for cytocentrifuged specimens and small volume such as cornea
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?

20 microns
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?______________________
Acanthamoeba cornea biopsy and culture

Broth culture showing spikes

E. coli overlay on non-nutrient agar