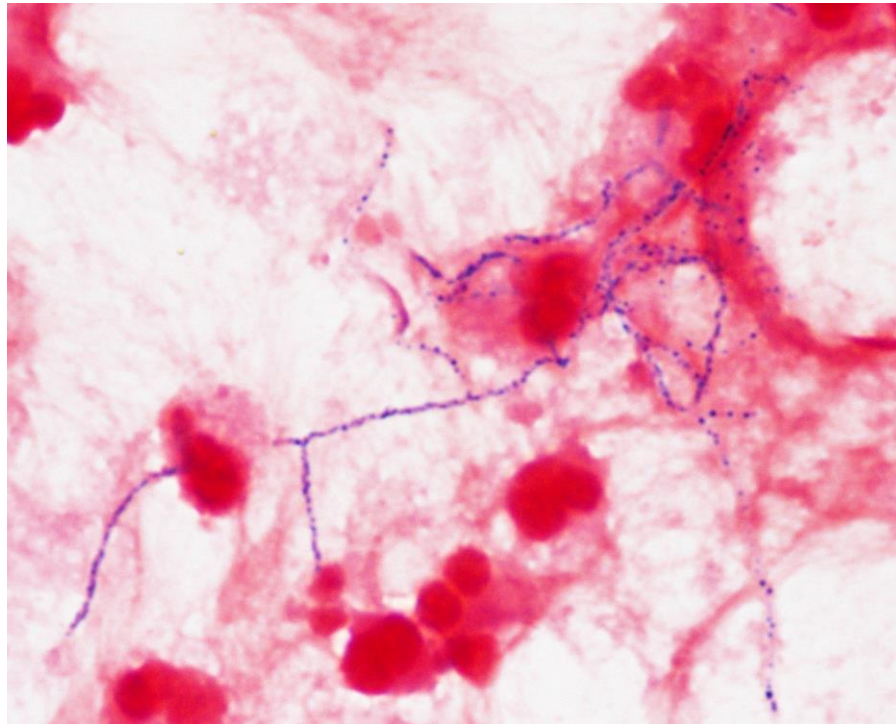


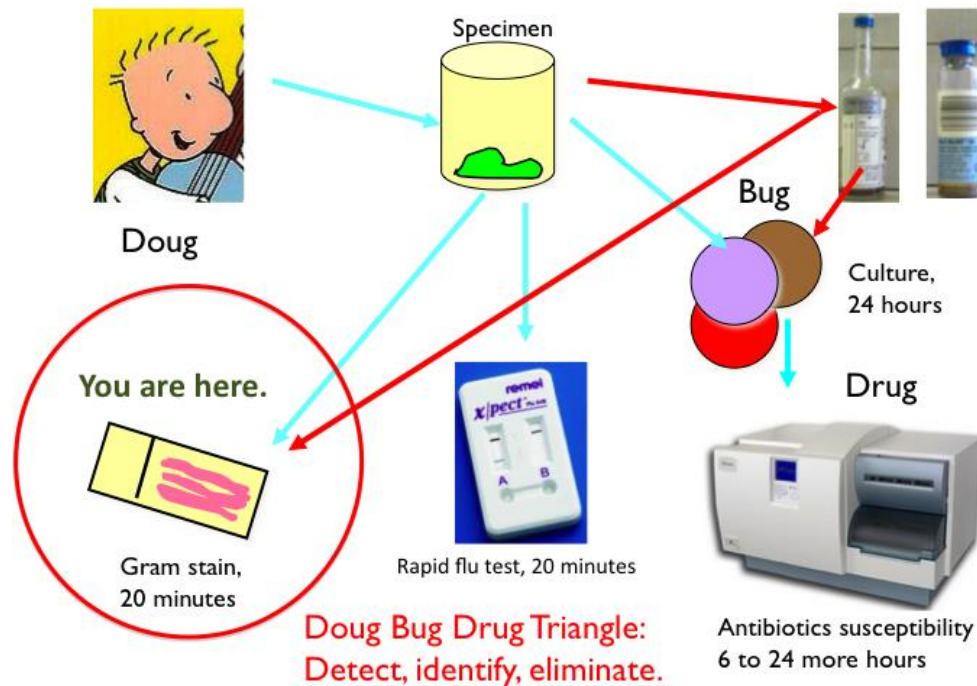
WS7: Optimizing Direct Specimen and Blood Culture Gram Stains SWACM Workshop 2016



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Objectives

- Optimize direct specimen and blood culture Gram stain quality by applying five keys in a case-based approach.
- Integrate the five keys into competency assessment.
- Implement a reasonable microscopy QA plan to satisfy CAP checklist requirements.
- Review the role of the positive blood culture Gram stain in the context of blood culture real time molecular tests.



The starfish story

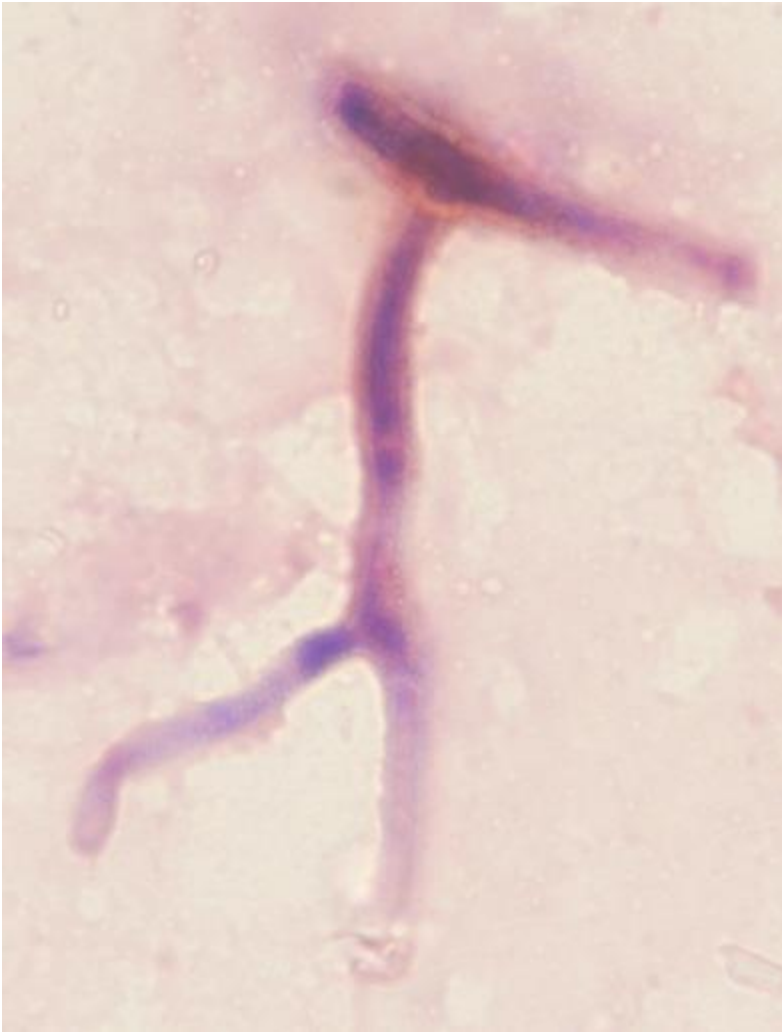
- Three yr old female: chemo for ALL
- Rt knee lesion, one week ago, now three new 0.5 cm lesions, rt forearm, rt leg
- Biopsy (not swab) from 1.0 cm lesion on rt knee, submitted for histology, routine, fungal, AFB cultures
- Original STAT gram stain reported as “no organisms seen”
- Pedi ID physician requests Gram 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.”

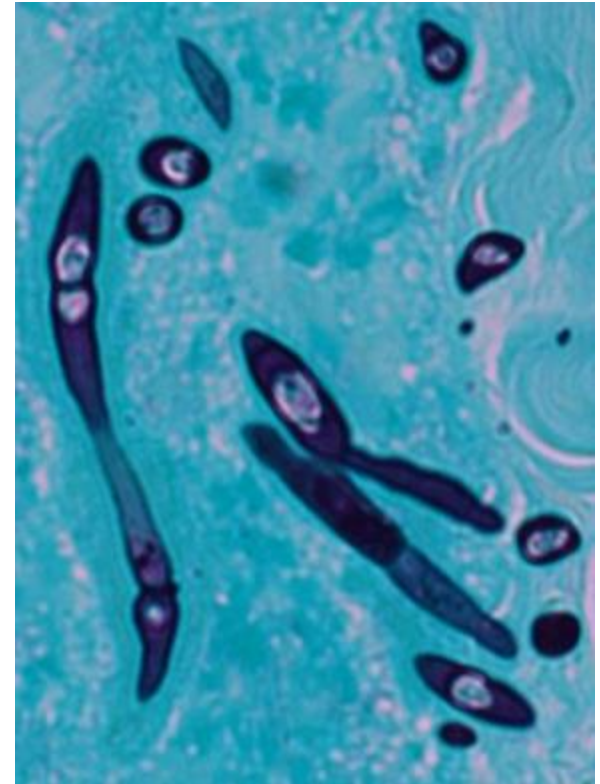
If you make enough differences, you make a sameness!

Knee tissue, what is it?



And the answer is?

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



GMS stain of same specimen

Real time patient impact?

Gram stain is faster than histology or journey to fungus lab,
turning “unknowns into knowns”

Content in Context

- The Gram stain is still the best, fastest and cheapest STAT multiplex “sample to answer” microbiology test.
- Body site and patient history give the biggest hints as to what we might see.
- We almost never have the complete patient history, but in important cases, doc talk helps!

This is your reality, right?

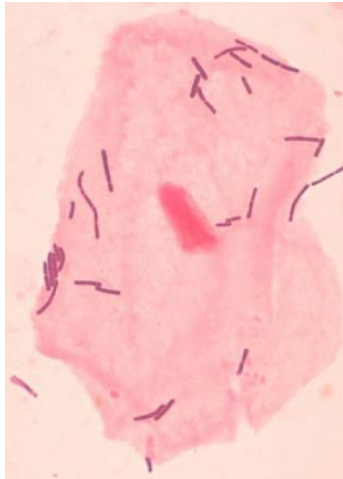
- Micro is slow: doctors are used to it and so are we! Are we accepting mediocrity?
- A lot of the specimens we get are substandard and nobody seems to care.
- It takes days, weeks, months to train a chemist but years to train a microbiologist!

So what are we gonna do about it?

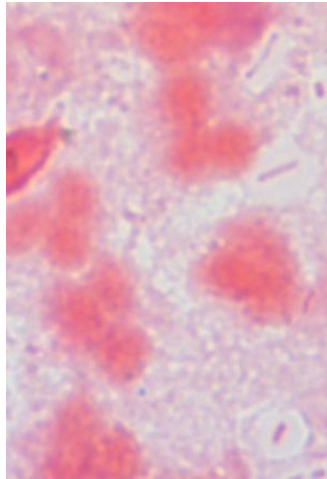
Basic review

- 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

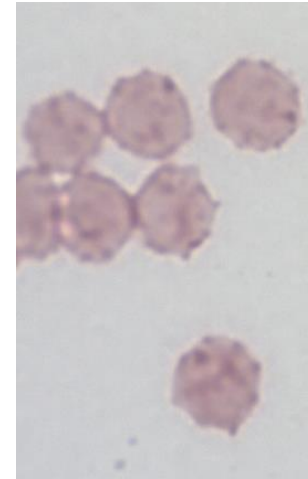
Cells in gram stains



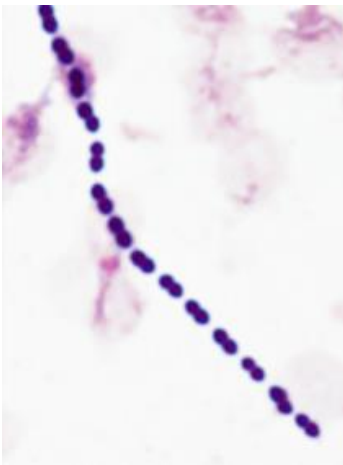
1 _____



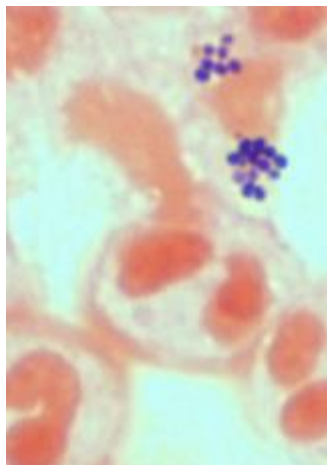
2 _____



3 _____



4 _____



5 _____



6 _____

Question:

The Gram stain can even detect:

- a) Microsporidia, *Acanthamoeba*
- b) *Chlamydia*, *Mycoplasma*
- c) *Mycobacteria*, *Nocardia*
- d) Both a) and c)

Question:

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:

Bingen, E., Lambert-Zechovsky, N., Mariani-Kurkdjian, P., Doit, C., Aujard, Y.,
Fournier, F. & Mathieu, H. (1990). Bacterial counts in cerebrospinal fluid of children with meningitis.
Eur J Clin Microbiol Infect Dis 9, 278–281.

Lauer, B. A., Reller, L. B. & Mirrett, S. (1981). Comparison of acridine orange and Gram stains for detection
of microorganisms in cerebrospinal fluid and other clinical specimens.
J Clin Microbiol 14, 201

- What are the sensitivity and specificity of specimen gram stain result correlated to culture growth results?
 - a) It depends on the study cited
 - b) These statistics cannot be calculated
 - c) 95% sensitive, 90% specific
 - d) 67% sensitive, 95% specific

Reference: **Sputum Gram's Stain in Community-Acquired
Pneumococcal Pneumonia
A Meta-analysis**

LTC WILLIAM W. REED, MPH, MC, USA; GREGORY S. BYRD, MD; and
COL ROBERT H. GATES Jr, MC, USA, Aurora, Colorado; ROBIN S. HOWARD, MA, Washington, DC; and
MICHAEL J. WEAVER, MD, Aurora, Colorado

Answer: it depends on study cited

- **Background:** The usefulness of sputum Gram stain in patients with community-acquired pneumonia (CAP) is controversial. The purpose of this study was to evaluate the usefulness of sputum Gram stain in etiological diagnosis and pathogen-targeted antibiotic treatment of CAP and HCAP.
- **Methods:** We conducted a prospective observational study on hospitalized patients with pneumonia admitted to our hospital from August 2010 to July 2012. Before administering antibiotics on admission, Gram stain was performed and examined by trained physicians immediately after sputum samples were obtained. We analyzed the quality of sputum samples and the diagnostic performance of Gram stain. We also compared pathogen-targeted antibiotic treatment guided by sputum Gram stain with empirical treatment.
- **Results:** Of 670 patients with pneumonia, 328 were CAP and 342 were HCAP. Sputum samples were obtained from 591 patients, of these 478 samples were good quality. The sensitivity and specificity of sputum Gram stain were 62.5% and 91.5% for *Streptococcus pneumoniae*, 60.9% and 95.1% for *Haemophilus influenzae*, 68.2% and 96.1% for *Moraxella catarrhalis*, 39.5% and 98.2% for *Klebsiella pneumoniae*, 22.2% and 99.8% for *Pseudomonas aeruginosa*, 9.1% and 100% for *Staphylococcus aureus*.
- **Conclusions:** Sputum Gram stain is highly specific for the etiologic diagnosis and useful in guiding pathogen-targeted antibiotic treatment of CAP and HCAP.

Validation of sputum Gram stain for treatment of community-acquired pneumonia and healthcare-associated pneumonia: a prospective observational study

Hajime Fukuyama^{1*}, Shin Yamashiro¹, Kiyoshi Kinjo², Hitoshi Tamaki¹ and Tomoo Kishaba¹

Definitions

- Analytical sensitivity: the smallest amount of an analyte that can be distinguished from background.
- **Sensitivity: positivity in disease, does the test detect all the true positives?**
- Calculation = $\text{true pos} / (\text{true pos} + \text{false neg}) \times 100$
- Analytical specificity: ability to detect only the analyte the test was designed to measure.
- **Specificity: negativity/normalcy in health, does the test detect all the true negatives?**
- Calculation = $\text{true neg} / (\text{true neg} + \text{false pos}) \times 100$

Compared to What?

If lab tests were pants

Sensitivity

How low can you go?



Specificity

Will you only detect gray high-waist pants and suspenders, narrow ties and white shirts on seemingly headless men?



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

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: the right 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 CAP MIC.22100 and MIC.22110

MIC.22100 Sputum Gram Stain

Phase I

A gram-stained smear is performed routinely on expectorated sputa to determine acceptability of a specimen for bacterial culture and as a guide for culture workup.

Evidence of Compliance:

- ✓ Policy defining acceptable specimens

MIC.22110 Unacceptable Sputum Specimens

Phase I

Specimens deemed unacceptable by Gram stain review are not cultured for routine bacteria (or cultured only by special request) and the health care provider or submitting laboratory is notified so another specimen can be collected without delay, if clinically indicated.

- Rejection criteria? CAP gives no definitive guidance!
 - Examine 20-40 fields on low power and average

# Epithelial cells/lpf	#PMNs/lpf	Interpretation
>10	>25	Acceptable
>10	<25	Unacceptable
<10	>25	Acceptable
<10	<25	Acceptable

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

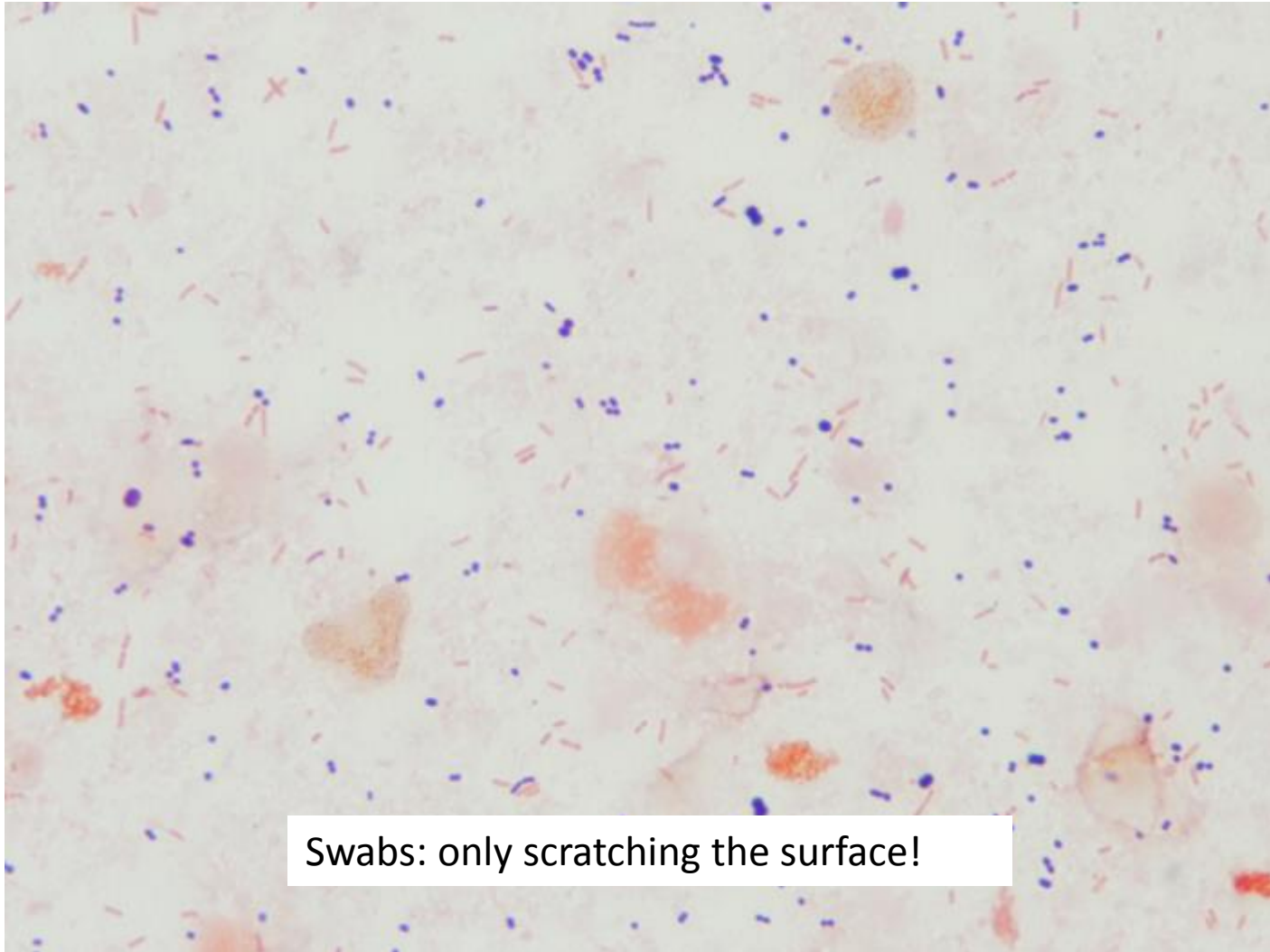
- Standard swabs absorb about ____? microliters and hold ____?% of what they absorb.
 - Vortex swab in .5 ml sterile saline before Gram stain and plating – but now I have another tube!
- Use new flocked swab collection kits available from multiple manufacturers.
- Gram stains suffer from inconsistency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



Flocked swab advantages

- Gets more specimen
- Releases more specimen
- Makes great cytoentrifuge Gram stains
- Can be used for anaerobes
- They work on automated platers
- Use the eluted liquid to inoculate media instead of the swab, gives you more specimen and some standardization.

Gram stain from swab, decubitus

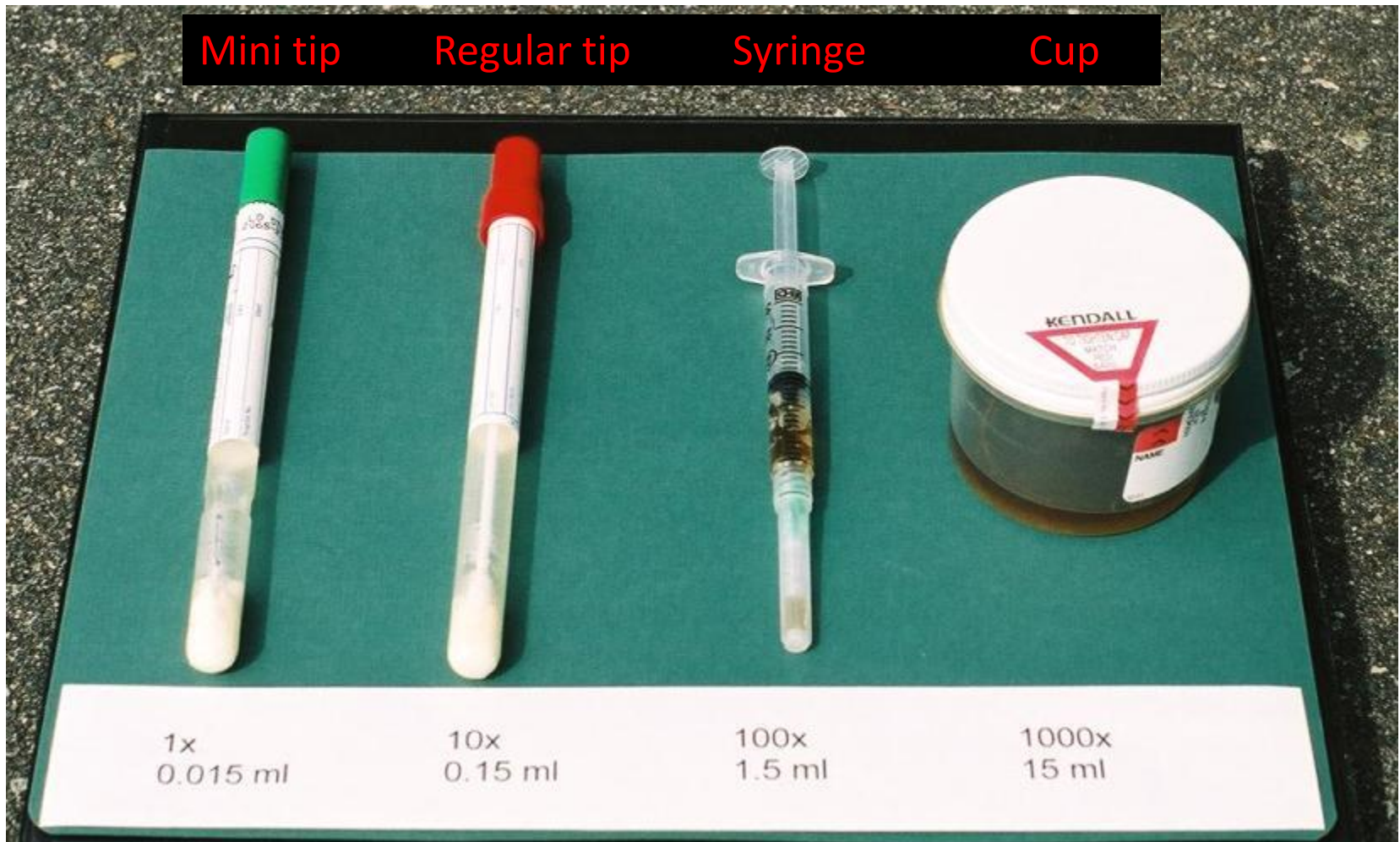


Swabs: only scratching the surface!

Repeat Gram stains from 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!

Teach nurses not to use swabs



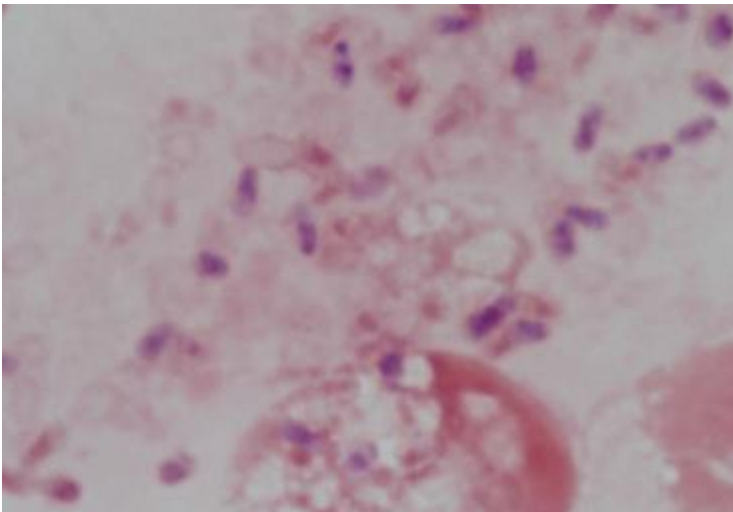
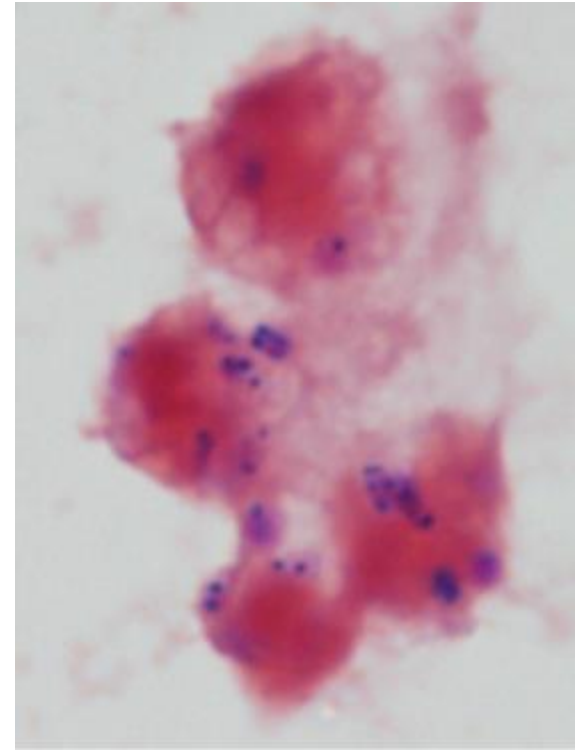
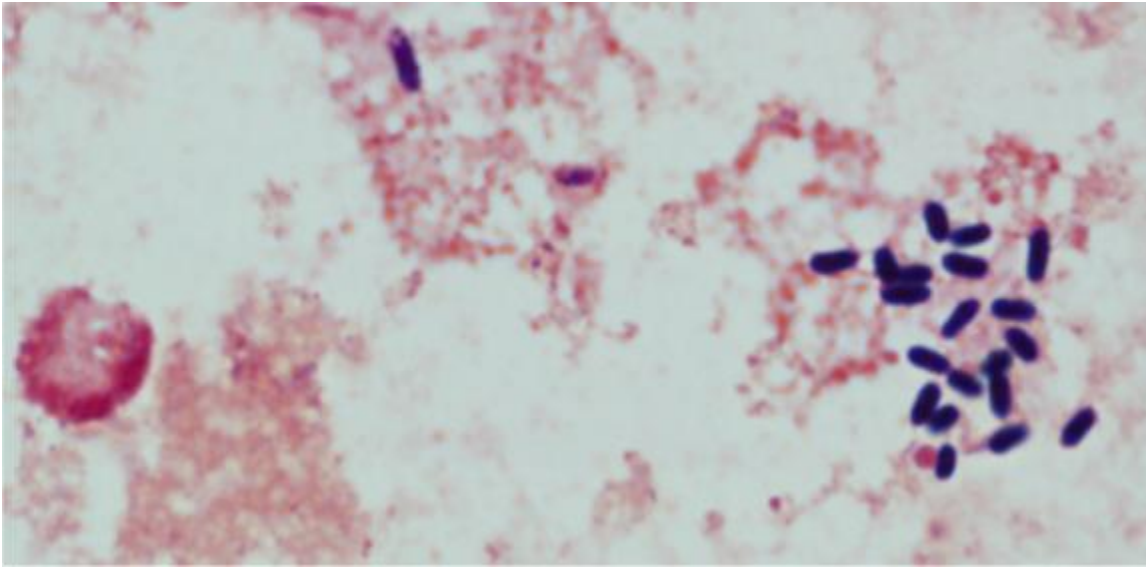
Should we ever Gram stain urine or stool?

- 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?

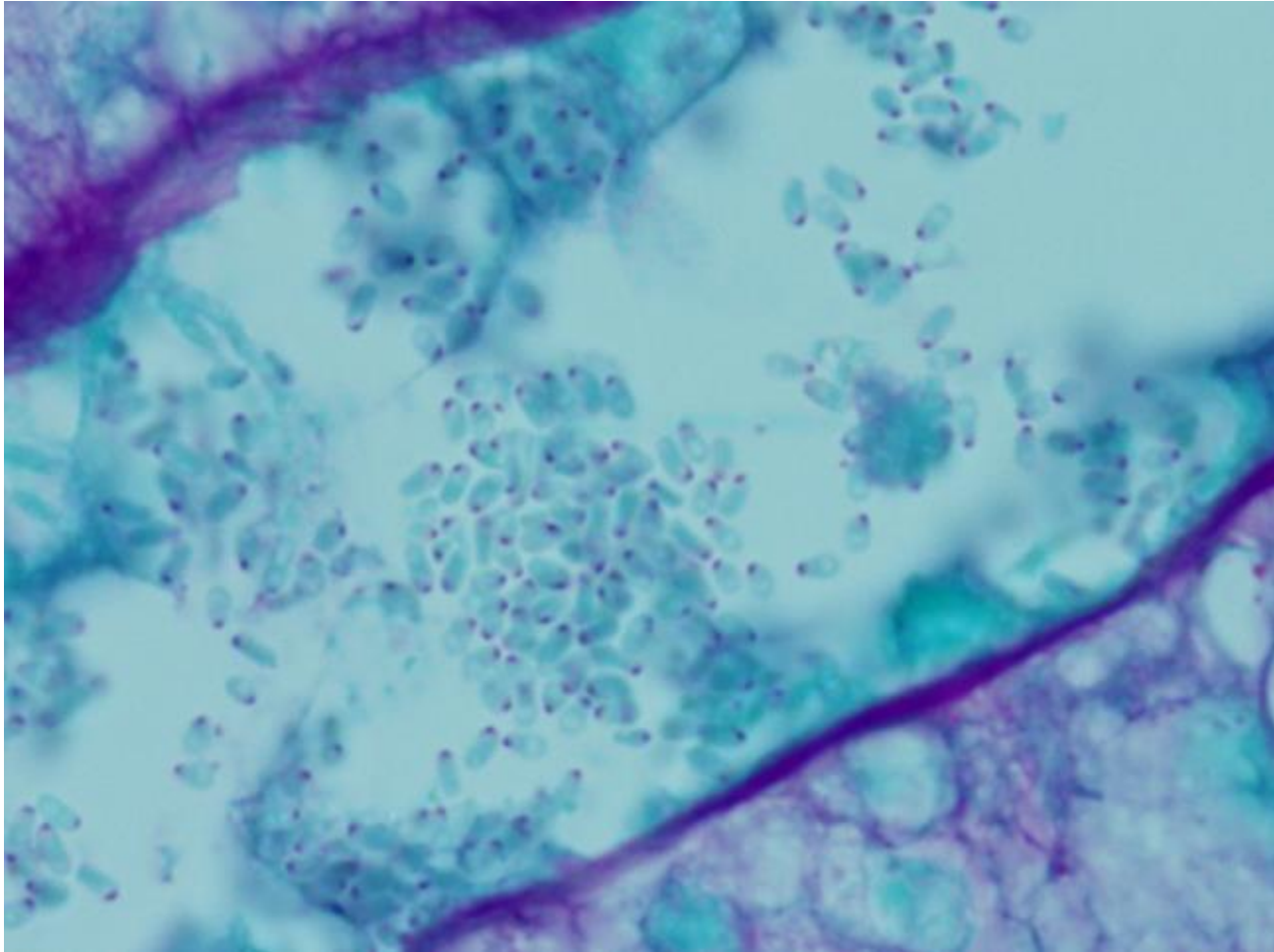


And the answer is?

- a) Microsporidia
- b) Anaerobic gram pos rods
- c) *Propionibacterium* species
- d) Yeast

Real time patient impact?

Organisms seen by pathologist in kidney biopsy, PAS stain



Key 1 summary

- Minimize use of swabs through education and good collection manuals for nurses
- Use every available opportunity you have to teach individual doctors, nurses, surgery, ED, etc., about specimen quality and quantity
- Use flocked swabs, liquid based collection systems.