What did the doctor say?
Key 5: reporting results
Rare, few versus 1+, 2+

- Pick one, but use the same one to enumerate grams and cultures.
- There is no correlation of numbers with disease severity and no scientific basis for enumeration!
- 1+, 2+, 3+, 4+
- Rare, few, moderate, many
- Doctors often think that “rare” means unusual!
- How does your UA dept quantify the urine microscopics?
Key 5: reporting results

• Human cells first, then bacteria most to least
• Code words may be meaningless to physicians
• We can use qualifiers
  – Resembling, consistent with, probable, possible, suggestive of
• Examples:
  – From broth culture only
  – GPC in clusters: we know what that means
What does this phrase mean to physician?

- Gram negative diplococci?
  - If blood or CSF versus ear or respiratory versus genital?
Condensed soup

• Long, expansive, descriptive lists of each bug seen don’t mean much, and waste everyone’s time.
• They also waste computer space.
• I love this phrase:
• “Mixed gram positive and gram negative organisms, no predominant organism noted”
<table>
<thead>
<tr>
<th>Old way</th>
<th>New way</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 10 epithelial cells per low power field</td>
<td>less than 10 epithelial cells per low power field</td>
</tr>
<tr>
<td>less than 25 PMNs per low power field</td>
<td>less than 25 PMNs per low power field</td>
</tr>
<tr>
<td>moderate gram positive cocci in pairs</td>
<td>moderate gram positive cocci in pairs</td>
</tr>
<tr>
<td>few gram positive cocci in clusters</td>
<td>few mixed gram positive and gram negative flora</td>
</tr>
<tr>
<td>few gram positive rods in palisades</td>
<td>no predominant organism noted</td>
</tr>
<tr>
<td>few gram negative rods</td>
<td></td>
</tr>
<tr>
<td>rare gram positive cocci in chains</td>
<td></td>
</tr>
<tr>
<td>rare gram negative diplococci</td>
<td></td>
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<td></td>
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<tr>
<td>many gram negative rods</td>
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</tr>
<tr>
<td>rare gram positive rod</td>
<td>rare gram negative diplococci</td>
</tr>
<tr>
<td>rare gram negative diplococci</td>
<td>rare gram positive cocci in pairs</td>
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</tr>
<tr>
<td>CERNER CODE</td>
<td>TEXT</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>MIXGP</td>
<td>MIXED GRAM POSTIVE FLORA</td>
</tr>
<tr>
<td>RMIXGP</td>
<td>RARE MIXED GRAM POSTIVE FLORA</td>
</tr>
<tr>
<td>FMIXGP</td>
<td>FEW MIXED GRAM POSTIVE FLORA</td>
</tr>
<tr>
<td>MMIXGP</td>
<td>MODERATE MIXED GRAM POSTIVE FLORA</td>
</tr>
<tr>
<td>NMIXGP</td>
<td>MANY MIXED GRAM POSTIVE FLORA</td>
</tr>
<tr>
<td>MIXGN</td>
<td>MIXED GRAM NEGATIVE FLORA</td>
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<tr>
<td>RMIXGN</td>
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<td>FMIXGN</td>
<td>FEW GRAM NEGATIVE FLORA</td>
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</tr>
<tr>
<td>RMIXGPN</td>
<td>RARE MIXED GRAM POSITIVE AND GRAM NEGATIVE FLORA</td>
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<td>FMIXGPN</td>
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<td>NMIXGPN</td>
<td>MANY MIXED GRAM POSITIVE AND GRAM NEGATIVE FLORA</td>
</tr>
<tr>
<td>NOPRED</td>
<td>NO PREDOMINANT ORGANISM NOTED</td>
</tr>
<tr>
<td>SUGGH</td>
<td>SUGGESTIVE OF HAEMOPHILUS SPECIES</td>
</tr>
<tr>
<td>SUGGSP</td>
<td>SUGGESTIVE OF STREPTOCOCCUS PNEUMONIAE</td>
</tr>
<tr>
<td>SUGGST</td>
<td>SUGGESTIVE OF STAPHYLOCOCCUS SPECIES</td>
</tr>
<tr>
<td>SUGGM</td>
<td>SUGGESTIVE OF MORAXELLA OR NESISSERIA SPECIES</td>
</tr>
</tbody>
</table>

You will never get some techs comfortable enough to use these “suggestive of” comments
Graded gram stains?

- Vaginal for suspected BV, clue cells
  - Do you know your Amsel criteria from your Nugent score?

<table>
<thead>
<tr>
<th>Lactobacilli</th>
<th>SCORE</th>
<th><em>Gardnerella, Bacteroides</em></th>
<th>SCORE</th>
<th>Curved gram-negative bacilli</th>
<th>SCORE</th>
<th>Sum=<em>N-Score</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>30 or &gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-30</td>
<td>1</td>
<td>&lt;1</td>
<td>1</td>
<td>&lt;1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1-4</td>
<td>2</td>
<td>1-4</td>
<td>2</td>
<td>1-4</td>
<td>1</td>
<td>5</td>
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<tr>
<td>&lt;1</td>
<td>3</td>
<td>5-30</td>
<td>3</td>
<td>5-30</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>30 or &gt;</td>
<td>4</td>
<td>30 or &gt;</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

http://www.cmpt.ca/pdf_supplemental_critique_information/nugent_score_interpretation_apr_09.pdf

Just get the BD Affirm instead!
Vaginal wet prep is three in one!

- Probably better than Gram because you can see all three potential pathogens:
  - Candida
  - Trichomonas
  - Clue cells of BV
Wound cultures

• Q-Score, proposed by Bartlett in 1974
• Q234: easier
• Both basically an inverse relationship between PMN and SEC.
• Susan Sharp, PhD has lots of info on this.
• How many of you are doing this?
• Susan.E.Sharp@kp.org
Wound cultures

- ASM May 2013 Hot Topic Blog: Guidelines for Work Up of Wound Specimens
- https://clinmicro.asm.org/blog/?p=199
- Yvette McCarter, PhD, D(ABMM)
Blood cultures, need for speed

• Methanol fix blood culture gram stains
• See case
• See examples of same slide, stained at same time, heat fix versus methanol fix
• Fast blood culture gram stains make a difference!
This both bothers and encourages me!
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.
Fast gram stain report = less death!

Decreased Mortality Associated With Prompt Gram Staining of Blood Cultures

Joan Barenfanger, MD,1 Donald R. Graham, MD,2 Lavanya Koiluri, MD,3* Gaurav Sangwan, MD,3 Jerry Lawhorn,1 Cheryl A. Drake,1 Steven J. Verhulst, PhD,4 Ryan Peterson,6 Lauren B. Moja, PharmD,7 Matthew M. Ertmoed, PharmD,7 Ashley B. Moja,5 Douglas W. Shevlin, MD,1 Robert Vautrain, MD,1* and Charles D. Callahan, PhD1

Am J Clin Pathol 2008:130:870-876

Figure 1. Diagram of a study of turnaround time (TAT) of Gram stains. * Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus groups B and G, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens, Pseudomonas aeruginosa, and Candida albicans.
Night shift positive = more death!

Figure 2: Culture positivity and mortality. * $P = .0624$. 
QA/QM plan

• Why do you need a QA plan for Gram stains?
• QC is not QA. QA is not QI.
• You can use QC and QA to affect QI.
• QC is easy, especially if you use those pre-made control slides that always work.
• QA monitors the whole process, people, places, things, actions.
• Gram QA is a good ongoing ANALYTICAL monitor.
The laboratory has protocols in place to use Gram stain results to provide a preliminary identification of organisms, evaluate specimen quality when appropriate, and to guide work-up of cultures.

**NOTE:** The laboratory should have guidelines for the interpretation of the Gram stain reaction of the organism, morphology of the organism, and the quantification of organisms and cells. The protocol should address correlation of direct Gram stain results with final culture results.

This does not mean that interpretation of the Gram stain morphology suggesting a specific organism identification (e.g., gram positive diplococcic morphologically suggestive of pneumococcus) is required.

**Evidence of Compliance:**
- Written procedure for Gram stain (laboratories may use the correlation of Gram stain results with the final culture results as a component of the QC program)
About Gram QC?

• Which reagent fails QC the most often?

• Which reagent actually degrades the fastest?

• Which of the reagents actually react chemically during the stain?

• Which step requires the most finesse?
Consistency is lacking

- Collection: specimen versus swab of specimen
- Specimen: liquid, viscous, solid
- **Technique – this is where competency comes in**
- 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+
- **Direct correlation of error rates with tech desire and ability!**
Gram stain QA case

- 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
QA case, CSF
And the answer is?

A. Amorphous material
B. *Staphylococcus aureus*
C. *Staphylococcus epidermidis*
D. *Corynebacterium* species
Physician dialog

• The classic Monday morning 8:00 am scenario:
  – Doctor wants to know why the culture did not grow, **Real time patient impact?**
• Lack of correlation between gram stain and culture results?
• Lab should have a good gram stain review policy.
• Acridine orange stain can resolve iffy Gram stains, but requires fluorescent scope and expertise.
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 completely missing gram neg organisms.
Gram to culture correlation

Sputum GRAM:
Less than 10 epithelial cells/LPF
Greater than 25 PMNs/LPF
Many small gram negative coccobacilli consistent with *Haemophilus*

CULTURE: *Haemophilus influenzae*, heavy growth
Gram to culture lack of correlation

Sputum GRAM:
Less than 10 epithelial cells/LPF
Greater than 25 PMNs/LPF
Moderate gram positive cocci in pairs
Few gram positive rods

CULTURE:
*Serratia marcescens*, heavy growth
Normal respiratory tract flora, moderate growth
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
The culture reading tech:

- Must look at the gram stain results while reading the culture (prelim or final.)
- Cerner, Soft can catch gram to culture mismatch
- The culture reading tech has the responsibility to investigate the discrepancy
  - Start with looking at already stained slide, misinterpretation, bad technique, clerical error?
  - Then stain duplicate if needed
  - Then re-stain the specimen if needed.
Gram stain review case

• 21 year old male, outpatient
• Swabs labeled “Back” for routine wound culture
• Gram stain: Many PMNs, Many GPC in clusters suggestive of Staph
• Aerobic culture: no growth after 48 hours
Gram stain review case
And the answer is ______________? 

A. Nonviable *Staphylococcus* 
B. Slow growing *Staphylococcus* 
C. Anaerobic gram pos cocci 
D. Midget yeast
The rest of the story

• Importance of Gram stain correlation
• Importance of anaerobic culture
Gram stain review policy, two extremes

– 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?

Duplicate slide and slide retention policy:
keep stained and unstained slide for 7 days, at the microscope
Practical Slide retention policy

• Keep stained and unstained duplicate 7 days.
• Have a written policy that says this.
• File them by day stained, Sun-Sat boxes for easy retrieval and review.
• Keep them as close to microscope as possible
• Keep any unusual keepers for teaching
  – coverslip using #1 thickness coverslip 24x50 size using Permount, can remove oil with xylene before coverslipping, but not necessary, just blot of excess oil and then add Permount on top, looks just fine.
  – Allow coverslipped slides to dry for a week before storing in vertical boxes or coverslips will creep and stick!
3. **POLICY**

3.1 When reading cultures, whether preliminary or final, it is the reading technologist’s responsibility to correlate the original clinical specimen Gram stain result with the growth on the plates and to take appropriate corrective action if the results do not correlate.

3.2 The original Gram stain slide should be pulled and reviewed by the technologist reading the culture under the following two circumstances only:

3.2.1 Organisms seen in Gram stain but no corresponding growth on culture.  
This is a lack of specificity, see explanation below.*

3.2.2 Organisms **not seen** in Gram stain, but heavy growth on culture.  
This is a lack of sensitivity, see explanation below.**

3.2.3 Organisms not seen in Gram stain, but \(>100,000\) CFU/ml growth on quantitative culture. This a lack of sensitivity, see explanation below.**

3.3 Corrective action shall include some or all of the following depending on the unique situation:

3.3.1 A notation in the worksheet stating that the Gram stain was reviewed.
3.3.2 Re-plating the culture, including plating an anaerobic culture if necessary.
3.3.3 Correcting the chart-able patient Gram stain report in Cerner, if review shows different results.
3.3.4 Completion of appropriate QA form.

*Lack of specificity. Organisms seen in Gram stain, but no growth of corresponding organisms on plates. Possible reasons include: nonviable organisms due to antibiotic therapy or delay in culture, fastidious or anaerobic organisms which do not grow on routine media, or contaminant organisms from a dirty slide. Lastly, clerical or technical errors, (results entered on wrong slide) should be considered.

**Lack of sensitivity. Cultures are more sensitive than Gram stains. Often, organisms are not seen in Gram stain, but are growing on the plates. Generally this correlates to light growth, or up to moderate growth on the plates. This is most common when only a single swab is submitted, and the specimen is diluted by streaking to plates and broth before making the Gram stain. Lastly, it is possible that the tech performing the Gram stain made a technical or clerical error (overlooked the organisms in question or entered results on the wrong slide).
Gram Stain review criteria*

Category 1 LACK OF SPECIFICITY
Gram stain: Bug seen
Culture: no growth

Category 2 LACK OF SENSITIVITY
Gram stain: No bug seen
Culture: heavy/4+ growth (>100,000 for quantitative cultures)

Culture tech correlates Gram stain and final culture results, based on the two criteria above.

Results correlate.
No further action.

Results do not correlate.
Culture tech reviews slide.

Culture tech fails to correlate results.
1. Director initiates QA form.
2. Director forwards QA form to culture tech

Gram stain correct but does not correlate with culture.
Culture tech reports on chart: "Gram stain results are reviewed and are correct."

Gram stain incorrect
1. Culture tech corrects Gram stain results, with comment “this is a corrected report.”
2. Culture tech documents on QA form.
   Culture tech forwards slide to Gram stain tech.
3. Gram stain tech reviews slide and adds commentary to QA form.
4. Gram stain tech forwards QA form to director.
5. Director completes monthly and quarterly QI report.

Bug seen, no growth (category 1)
This is most critical discrepancy, requires investigation
1. Culture tech investigates, dead bug, anaerobe, fastidious, slow grower?
2. Culture tech re-Gram stains original specimen with fresh slide, re-plates for anaerobes, replates original culture if indicated, varies with individual case.

No bug seen, heavy/4+ growth (category 2)
No further action.

*Some possible exceptions to review policy:
Sputum, endotracheal, vaginal and other specimens which contain mixed flora (including anaerobes) may be problematic.
Gram stains with no predominating organism and/or cultures with only normal flora in these body sites generally do not require review.
Proficiency versus Competency


• Six elements specified by CLIA

• In CAP lab general checklist, Micro

• See good reference below
CAP checklist: Morphologic Observation MIC.11350

MIC.11350  Morphologic Observation Assessment  Phase II

The microbiology laboratory at least annually assesses morphologic observations among personnel performing Gram, trichrome and other organism stains, to ensure consistency.

NOTE: Suggested methods to accomplish this include:

1. Circulation of organisms with defined staining characteristics, and/or
2. Multi-headed microscopy, and/or
3. Use of photomicrographs with referee and participant identifications (e.g. former CAP microbiology Surveys or other photomicrographs from teaching collections)
4. Use of digital images

Evidence of Compliance:
✓ Written procedure defining the method and criteria used for evaluation of consistency AND
✓ Employee records documenting morphology assessment

REFERENCES
1) Flournoy DJ. Interpreting the sputum gram stain report. Lab Med. 1998;29:763-768
Six elements

1. Direct observations of routine patient test performance, including patient preparation, if applicable, specimen handling, processing and testing
2. Monitoring the recording and reporting of test results
3. Review of intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records
4. Direct observation of performance of instrument maintenance and function checks
5. Assessment of test performance through testing previously analyzed specimens, internal blind testing samples or external proficiency testing samples
6. Assessment of problem solving skills
When and how often?

• Evaluating/documenting performance of individuals responsible for high complexity testing at least semiannually during first year the individual tests patient specimens. Thereafter, evaluations must be performed at least annually unless test methodology or instrumentation changes, in which case, prior to reporting patient test results, the individual’s performance must be reevaluated to include the use of the new test methodology or instrumentation.
On-line competency assessment

- University of Washington
2nd/3rd shift competency

• Shift generalists are **not** naturally curious about what grew from the gram stains they were FORCED to do last night! Ironically techs that get most important gram stains are least experienced.

• Monthly gram stain challenge!

• Shift techs record the accn numbers of five slides they performed per night and look up the culture results to verify correlation.

• Manager/director reviews results, discuss any discrepancies, provide positive feedback.

• Be willing to come in to teach on shift!
How to get enough specimen?

• If you use the same specimen for everyone they will figure it out.

• Simulate a fake positive CSF or use REAL recent positive blood cultures, use real negative CSF – important to test negative specimens for competency.

• Fake positive CSF: Get a H. flu or S. pnuemo BAL and dilute it 1/10 with saline, then preserve by mixing this dilution 1/1 with cytology fixative (ThinPrep vial)
  – Formalin is no good, causes WBCs round up and clump.
Competency interventions

• How many errors can you have per month?

• What if a tech fails competency?

• How do you maintain confidentiality and respect?
Good references
Others

• Barenfanger, J, Drake, C. Interpretation of Gram Stains for the Nonmicrobiologist. Laboratory Medicine July 2001, number 7, volume 32


• Munson, E., Block, T., Basile, J., Hryciuk, J., Schell, R. Mechanisms to Assess Gram Stain Interpretation Proficiency of Technologists at Satellite Laboratories, J Clin Micro, Nov 2007 3754-3758
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, competency testing and review can improve processes.
• Improvements such as flocked swabs, limiting use of swabs, cytocentrifugation and methanol-fixation can improve quality.
• May have immediate real-time patient impact, guiding diagnosis and therapy.