The Gram Stain – What it can do for you!

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No disclosures
Is the Gram Stain Helpful?

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- Assess specimen quality
- Diagnostic & therapeutic value
- Culture correlation
- Predictive value
- Cost effective
- Positive patient outcome
- Healthcare benefits

Gram stain (If done well)
Health Care Challenges

- Micro lab consolidation trend – core lab.
- Satellite lab generalists responsible for critical Gram stains (GS): CSF, blood, sterile fluids, tissues.
  - Require training, competency, support and feedback.
- CAP requirements for GS consistency, correlation.
- Maintain communication with off-site health providers.

Susan Sharp, Elder, et al., Comp Assessment in the Clin Micro Lab, Clin Micro Reviews, July 2004
Church, Deirdre et al, Quantitative GS Interpretation Criteria Used by Micro Labs in Alberta, Canada, JCM Nov 2000, vol 38, no 11, 4266-4268.
Gram Stains at Multiple Sites
Key to Success: Partnership

Hospital A
Hospital B
Clinic E
Core Micro Lab
Stat Lab D
Hospital C

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Challenges of Reading Gram Stains by Non-Microbiologist

- Less expertise:
  - confidence
  - consistency
  - competency
  - correlation

- How to improve GS proficiency?


1st Scenario

Generalist at satellite lab

NICU blood culture GS

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Blood Culture = viridans Strep

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55 year old female ER patient with flank pain. 
**Dx:** r/o kidney stone. 
**Previous UTI with Proteus.** 
**On antibiotic therapy.** 
**Positive blood culture.** 
**Gram stain = ??**
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Objectives

- Review the diagnostic and therapeutic impact of the Gram stain (GS).
- Review tips for a high quality GS.
- Maximize clinically relevant, accurate GS results:
  - Report to probable genus level.
- Meet CAP requirements for GS:
  - Consistency and correlation.
- Utilize telemicroscopy.
- Provide positive patient outcomes!
Inadequate Diagnostics

“...More often, physicians must use incomplete or imperfect information to diagnose an infection and thus prescribe an antimicrobial just-in-case, or prescribe a broad-spectrum antimicrobial when a specific antibiotic might be better. These situations contribute to selective pressure and accelerate antimicrobial resistance.”

2008 www.NIH.gov
Diagnostic Role of GS

- Remains integral tool in micro and infectious disease.
- Infectious disease among top 3 causes of death in US.
  - Hospital acquired infections: 100,000 deaths, $6 billion.
  - CA-MRSA: 89,000 cases annually, $8 billion.
  - Antibiotic resistance is among top 5 public health care concerns: 2 million illnesses, 23,000 deaths annually.
- CDC plan: Target pathogen, treat infection, not contamination or colonization.
- GS can help target pathogen and treatment!

Antibiotic Resistance Threats in the US 2013. Executive Summary, CDC HHS.
Diagnosing and Treating BSI

- Up to 40% of all patients with blood stream infections receive inadequate antibiotic treatment until the 1st notification of a positive blood culture…GS.
  - 10-20% of patients not started on any antibiotics until GS.
  - 30-45% require change in empirical treatment.
- Blood GS reported in <1 hour can lead to 17% lower mortality.
- GS accuracy critical for accurate therapy, less mortality, saves $.

Blood Culture GS Diagnosis?

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Diagnosing Bacterial Meningitis

• CSF is one of the most important GS in Micro.
  • Rapid, accurate ID of the pathogen in 60%–90% of patients with community-acquired bacterial meningitis,
  • 97% specificity
    • Depends on pathogen and prior treatment.
    • Cytospin can increase sensitivity 100x.
  • Spend extra time searching for pathogen if WBCs present – can be sparse. Consider acridine orange.

Baron E. et al., IDSA and ASM Guidelines 2013.
Tunkel, Hartman, Kaplan et al., CID 2004:39, 1 Nov. 1269-70
CSF GS Diagnosis?

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Diagnosing Fungal Infections


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Diagnosing BV

- GS is “Gold Standard”, more specific than culture or probe.
- Requires pattern recognition, compare ratio of normal (Lactobacillus) to altered flora (Gardnerella, Mobiluncus, other anaerobes).
- CAP requirement MIC.22280: detection of Bacterial Vaginosis by “Graded Gram stain” for evaluation of vaginal flora.
- NIH recommends screening in high risk pregnancy.
- Underutilized.

MMWR CDC Sexually Transmitted Diseases Treatment Guidelines, 2010. [www.cdc.gov/std/treatment](http://www.cdc.gov/std/treatment)
IDSA and ASM Guidelines 2013, Baron et al., CID 2013;57.
Hammoud, K., Treatment of GU Tract Infections: An Evidence Based Approach. 4-13-2012.
Carol Spiegel, Bacterial Vaginosis, Clinical Micro Review. 1991;4:485-502
Nugent, Krohn Ability of diagnostic procedures to evaluate one阴道 anaerobic bacterial infection in women, JCM. 1991;29:297-301.
Graded Gram Stain

Normal

Abnormal (BV)

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Quality is Not Optional

Anything less than accurate, clinically relevant results “is below the community standard of care.”

Quality Specimen + Quality GS + Competent Tech = Accurate Results
GS Tips

Evaluate specimen.
Avoid contamination, sterilize slide.
Cytospin fluids.
Make 2 smears.
Avoid Gram-variability:
- Methanol fixation – no heat.
- Reagent concentration
  Iodine
  Decolorizer
Make reference slide set.

Garcia L et al., Clin Micro Proc Handbook, Chap 3, ASM.
Chapin-Robertson et al., Cytospin Increases Sensitivity… of
Zuchowski, L., GramStainology™: Gaining Proficiency in
Diagnostic Interp and Results Reporting, ASCP WLP 2015.
Illustration by Kelly Zubeck.
Stain Quality Issue

Overdecolorized cocci? Gram-variable Bacillus?
Gram Stain Quality – Wow!

Heat fixed

Methanol fixed

Zuchowski, L., GramStainology™: Gaining Proficiency in Diagnostic Interpretation and Results Reporting, ASCP WLP 2015.

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CSF Cytospin: Bad and good

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Clinically Relevant Reporting
Avoid Vague Reports

Blood culture GS: GPC and GNB.

Not helpful!

Report to genus level when possible to guide treatment.
Clinically Relevant Reporting

- “No information is better than misinformation.”*
- Too much info may lead to unnecessary treatment.
- GS should guide culture work-up.
- Describing organism genus more useful than morphology description.

*Raymond Bartlett, MD., Medical Microbiology: Quality Cost and Clinical Relevance, 1974
Baron E. et al., IDSA and ASM Guidelines 2013.
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Predictive Value of GNB

- Differentiation of GNB reliable:
  - *Hemophilus*— (in 10% of symptomatic patients)
    - Sensitivity 76%, Specificity 95-100% for gncb.
    - PPV 100%, NPV 96%
  - Enterics – 82% for blunt-ended gnb (68% prevalence).
  - *Pseudomonas* – 56% for slender, sausage-shape gnb (20% prevalence).
- Why report just GNB?

Describe “GNB”: Suggestive of Enterics, *Fuso, Hemo*
Describe “GPR” Suggestive of Clostridium/Bacillus, Branching, Diphtheria
Predictive Value of *Staph* or *Strep*?

- **Staph** – 98% sensitivity and 100% specificity for GPC in grapelike clusters.
- **Strep** – 100% sensitivity, 98% specificity for GPC in pairs and chains.
- **Strep pneumo** – 75% sensitivity and 97% specificity.
- **Gram stain** gave presumptive diagnosis for 80% of good quality specimens.

**Why report just “GPC”?**

Aggar, Maki, et al., Efficacy of direct Gram stain in differentiating Staph and Strep in blood cultures positive for GPC. JCM 1978.
Describe “GPC” Suggesting Staph, Strep or Strep pneumo

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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**
GS Consistency Challenges:

- Variability
  - Non-standard specimen
  - Smear, stain quality
- Subjectivity
  - GS interpretation.
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.

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)
Culture Correlation - Accuracy

- Depends on GS quality and expertise.
- Never 100%, but up to 97% for proficient techs.
- 99.3% for blood cult GS read by experienced techs.
  - 57 of 8,253 blood cult GS misread in 2 years=0.7%*
- 50% sputum cult clinically misleading without GS correlation.
- Target pathogen! Appropriate monotherapy 94% of time when guided by GS.


GS Correlation

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GS Correlation

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GS Correlation QA

- Include in QA policy. (CAP MIC.21530)
- Monitor extreme discrepancies:
  - Negative GS, but positive culture
  - Positive GS, negative culture
- Bench tech must correlate results.
- Follow up, GS review, feedback.
- Having Micro tech review previously read slides is best indicator of the tech’s GS interpretation proficiency.*
- Consider telemicroscopy…

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Telemicroscopy

- There is growing interest for rapid, remote, expert consultation.*
- Easy and cost effective.
- Allows real-time slide review with experts 24/7.
  - Builds confidence for non-micro techs, beginners.
  - Improves competency, accuracy, correlation.
- Evidence based, increased interpretive reporting.
- Email or print images, create image library.


Telemicroscopy Advantages

- Bioterrorism preparedness (alternative to STATPack™)
- Public health consultation – share parasite images [www.cdc.gov/DPDx](http://www.cdc.gov/DPDx) (e.g. *Cyclospora* outbreak 2013).
- Boost QA program to meet CAP standards:
  - Improve consistency and correlation at satellite labs.
- Allows satellite labs to keep blood cultures on-site.
- Contributes to positive patient outcome.
- Benefits entire health care system.


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Telemicroscopy: QA Plan to Improve GS Proficiency in Satellite Lab

- Review each GS daily or in real time with core micro lab.
- Evaluate slide/stain quality and interpretation.
- Track correlation – accuracy rate.
- Monitor revised reports – provide feedback.
- Maximize GS results! (“GrainStainology™ 2.0”)
Telemicroscopy Success

- Improved accuracy to >97% for 1000 slides!
- Increased confidence among non-micro techs.
- Results maintained since 2011.
- Rare revised reports!
- Win-win!

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Share Expertise!

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Smart Phone Option

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Slide Practical
Abscess Drainage GS
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Sputum GS

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Anaerobic Blood Culture GS

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Summary: What Can the Gram Stain Do for You?

• Assess specimen quality.
• Provide accurate, clinically relevant results.
• Support or change diagnosis and therapy.
• Provide mechanism for culture correlation.
• Telemicroscopy - bridge gap with satellite labs.
• Contribute directly to positive patient outcomes.
• Save health care costs. Win-win!
• What can you do to maximize your next GS?
Thank you!

Questions?

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