Challenging Gram Stains

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No disclosures

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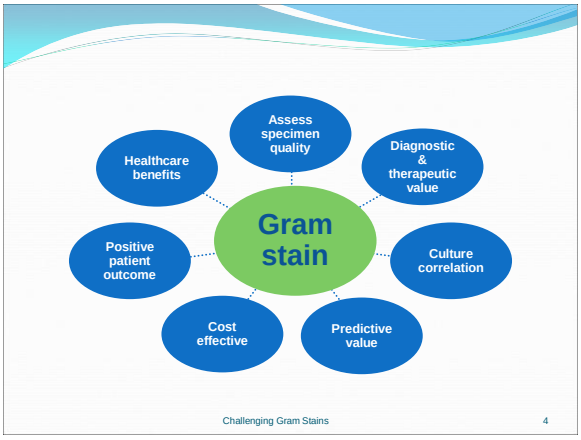
Objectives

- Recognize challenges that impact quality of Gram stains (GS).
- Identify GS quality processes to overcome challenges, and maximize positive patient outcomes.
- Review case studies utilizing telemicroscopy to support or change diagnosis and treatment options for infectious disease.

Role of Gram Stain

- Integral tool in microbiology 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!**

Brinsley K et al. A J Infect Control 2005;33(1) 53-54.
Tom Frieden, Director CDC 2014. www.cdc.gov/mrsa/statistics/index/html accessed 4/14/14.
Antibiotic Resistance Threats in the US 2013, Executive Summary, CDC HHS. www.nih.gov.



Quality is Not Optional

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

Baron, Ellen Jo, Miller, J Michael, et al. **IDSA Guidelines, A Guide to Utilization of the Micro Lab for Diagnosis of Infectious Diseases: 2013 Recommendations** by the Infectious Diseases Society of America and ASM. CID 2013:57 August 15.

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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

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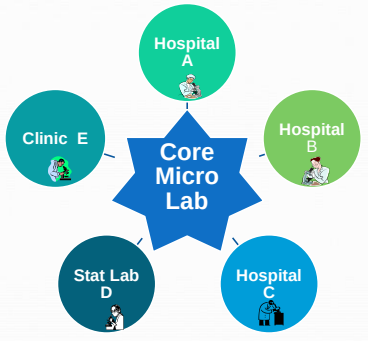
Health Care Challenges Impact GS

- Affordable health care, financial restrictions.
- Micro lab consolidation trend – core lab.
- Satellite lab generalists responsible for critical Gram stains: CSF, blood, sterile fluids, tissues.
  - Less expertise
  - Lower GS accuracy and correlation rate
  - Less communication with off-site providers, labs
  - Standard of care required.

Sautter Robert, Thomson Richard Jr, Consolidated Clinical Micro Labs, JCM, May 2015 vol. 53 no.5 1467-1472.
Ansara MK, *Experience and recommendations for consolidating a micro lab Clin Microbiol Newsl. 2002;24(3):17-23*
Barenfanger, Joan, et al., *Interp of GS for the Nonmicrobiologist*, LabMed, July 2001, No 7, Vol 32..
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



Technical Challenges

- Specimen collection and transport
  - Best practice guidelines prevent compromised quality
    - Collect sample prior to antibiotic therapy
    - Clean wound site prior to collection, minimize contamination
    - Fluid and tissue better than swab
    - Prevent transport delays
- Processing sample, making quality smear, stain.
- Evaluating, interpretting and reporting stained smear
  - Recognizing normal flora versus pathogens,
  - Avoiding artifacts, identifying unusual organisms.

Goal: Overcome GS Challenges

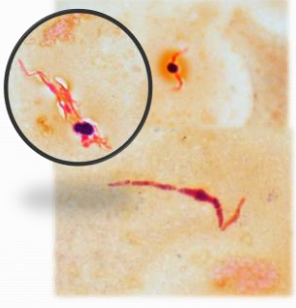
- Provide accurate, clinically relevant results
- Attain CDC goal of targeting pathogen, therapy
- Meet CAP requirements for GS
- Meet community standard of care,
- Maximize positive patient outcomes
- Save health care costs.

Implement GS Quality Plan

- Measure accuracy rate for baseline, set goal
- Improve GS quality and expertise.
- Partner with core lab
- Enhance training and competency program
  - Increase frequency of competency exercises
  - Review slides with major discrepancies
  - Provide feedback
- Consider telemicroscopy.

1st Case Challenge

55 year old female patient with flank pain, community hospital ER.
Dx: r/o kidney stone.
Previous UTI – *Proteus,* on antibiotic therapy.
Positive blood culture
Gram stain = ??
Telemicroscopy review



Antibiotic Effect

- Sub-inhibitory concentrations of antibiotics can produce abnormal, pleomorphic bacteria morphology.
- Penicillin can cause:
  - Gnrs to elongate and produce filaments
  - *Pneumococci* to form rods or globules
  - *Staphylococci* to enlarge and become irregular
  - *Gonococci* to form globules
- Body fluid antibiotic levels can cause effect.
- Gram stains challenging.
- Collect specimens prior to therapy.

Lorian et al., "Abnormal Forms of Bacteria Produced by Antibiotics", AJCP, Vol 64, Nov 1975.

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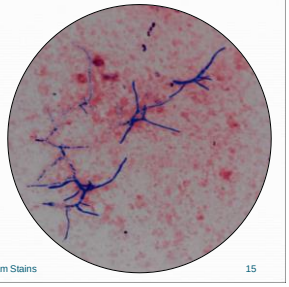
2nd Case Challenge

- Elbow abscess GS= rare WBC, no organisms.
  - Aerobic and anaerobic culture final = **No growth**.
- Physician questioned negative results**.**
- Patient Chart review:
  - Day 1 - Pt admitted, elbow infection, Clindamycin initiated.
  - Day 2 - Levofloxacin added.
  - Day 3 - Ertapenem and Vanc started.
  - Day 4 - Elbow abscess drained for C&S.
- Collect specimens prior to antibiotic therapy!

3rd Scenario

Pleural fluid smear too thick, no organisms seen.

***Nocardia* grew on culture.**



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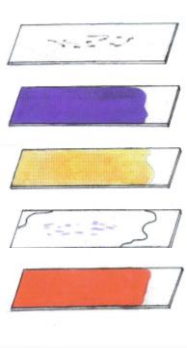
Quality Specimen + Quality GS + Competent Tech = Accurate Results

Stain Quality

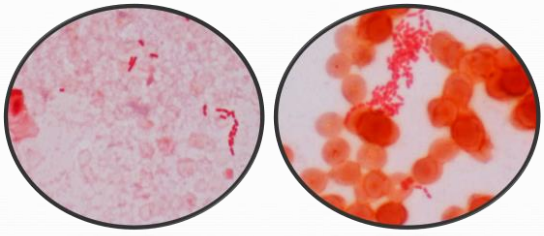
Evaluate specimen.
Avoid contamination, sterilize slide.
Cytospin fluids.
Make 2 smears.
Avoid Gram-variability:
-Methanol fixation – no heat!
- Counterstain longer for anaerobes
-Reagent concentration
Iodine (non-stabilized)
Decolorizer



Garcia L et al., Clin Micro Proc Handbook, Chap 3, ASM.
Chapin-Robertson et al., Cytospin Increases Sensitivity… of CSF, JCM 30:377-380, 1992.
Zuchowski, L., GramStainology™: Gaining Proficiency in Diagnostic Interp and Results Reporting, ASCP WLP 2015.
Illustration by Kelly Zubeck.

Gram Stain Quality – Wow!

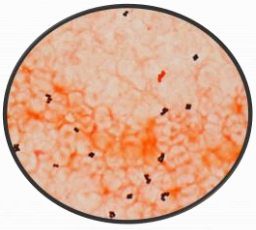
Heat fixed **Methanol fixed**



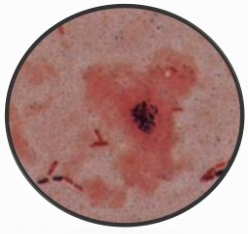
Zuchowski, L., GramStainology™: Gaining Proficiency in Diagnostic Interpretation and Results Reporting, ASCP WLP 2015.

Stain Quality Issue

Overdecolorized cocci?



Gram-variable Bacillus?



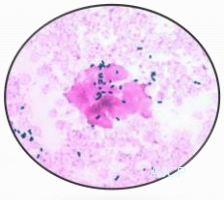
Errors in Interpretation: Human Error and Cell Wall Changes

- 57 of 8,253 positive blood culture gram stains were misread in 2 yr period = 0.7%.
- 0.1% were Gram neg organisms staining gram positive (*Acinetobacter*).
- 1.3% were Gram pos organisms staining gram neg (*Bacillus* and *Clostridium*).
- Gram variable staining due to cell wall changes with loss of viability.

Rand, Kenneth et al., Errors in Interpretation of Gram Stains from Blood Cultures, AJCP, 2006;126:686-690. Dept of Path and Lab Med, Div of ID, Univ of FL,Gainesville.

Errors in Interpretation of Gram Stains from Blood Cultures

***12% Acinetobacter* stain Gram positive**



***8% Bacillus* species stain Gram negative**

Rand, Kenneth et al., Errors in Interpretation of Gram Stains from Blood Cultures, AJCP, 2006;126:686-690. Dept of Path and Lab Med, Div of Infectious Disease, Univ of FL, Gainesville

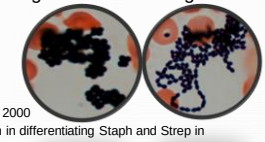
Clinically Relevant Reporting

- "No information is better than misinformation."*
- **Describing organism genus is more useful than just morphology description.**
- Avoid vague GS results: GPC, GNB
- GS should guide culture work-up.

*Raymond Bartlett, MD., Medical Microbiology: Quality Cost and Clinical Relevance, 1974
Baron E. et al., IDSA and ASM Guidelines 2013.
Bartlett R et al, Interp and Reporting of Organisms in Direct Smears, 1982. JAMA 247:857-59.
Bartlett R.,et al. 1991. Diagnostic Microbiology. Infect Disease 14:195-201

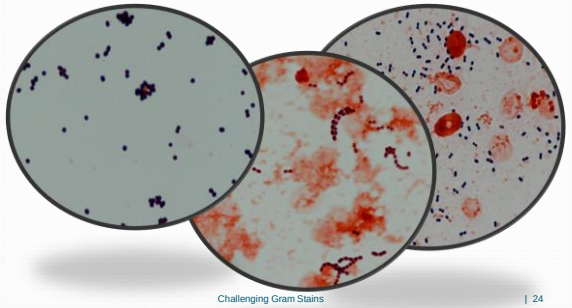
Predictive Value of *Staph* or *Strep*

- *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"?**

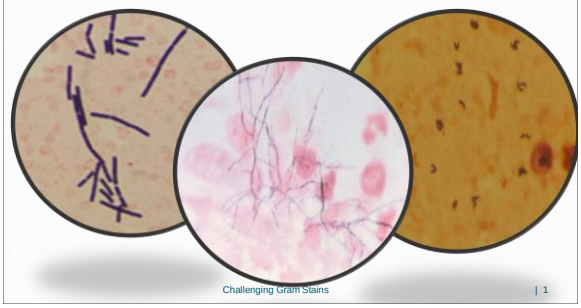


Roson, B, et al., Clin Infect Disease 31:869-74, 2000
Aggar, Maki, et al., Efficacy of direct Gram stain in differentiating Staph and Strep in blood cultures positive for GPC. JCM 1978

Describe"GPC" Suggesting *Staph, Strep* or *Strep pneumo*



Describe“GPB” Suggesting *Clost/Bacillus*, Branching, *Diphth*

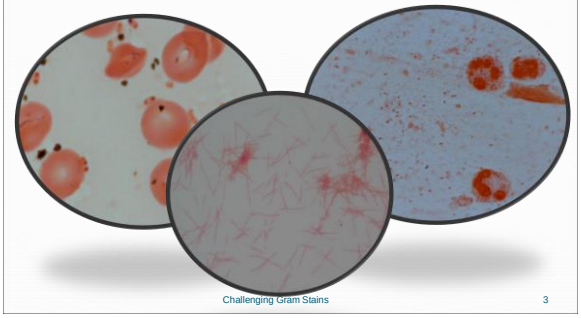


Predictive Value of GNB

- Differentiation of GNB reliable:
  - *Hemophilus*–10% prevalence in symptomatic patients
    - Sensitivity 76%, Specificity 95-100% for GNCB.
    - PPV 100%, NPV 96%
  - Enterics – 82% for blunt GNB.
  - *Pseudomonas* – 56% for slender, sausage-link GNB.
- **Why report just GNB?** Target the pathogen.

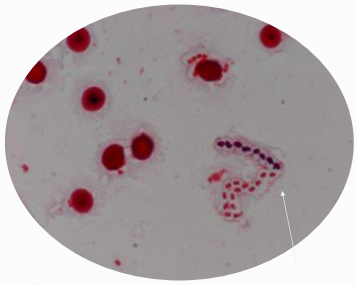
Sadeghi, E., Matlow, A., et al., Utility of GS of sputa in cystic fibrosis , JCM p 54-58, Vol 32, No 1, Jan 1994.
Bartlett R et al, Interp and Reporting of Organisms in Direct Smears, 1982. JAMA 247:857-59.
Bartlett R.,et al. 1991. Diagnostic Micro Infect Disease 14:195-201.

Describe “GNB”: Suggesting Enterics, *Fuso, Hemo*



Encapsulated GNB Challenge

Pantoea (Enterobacter) agglomerans



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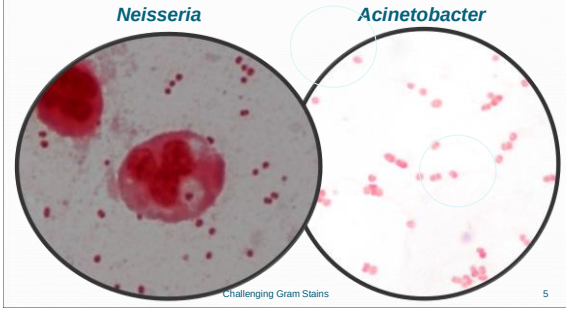
GNDC compared to short GNB

Neisseria *Acinetobacter*



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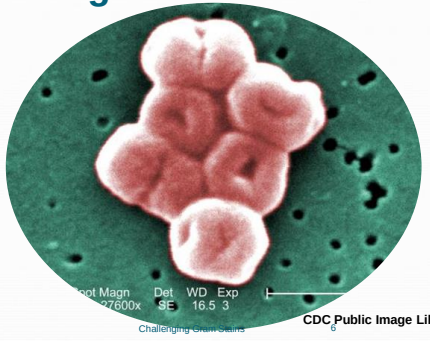
Short gnr - *Acinetobacter*



CDC Public Image Library

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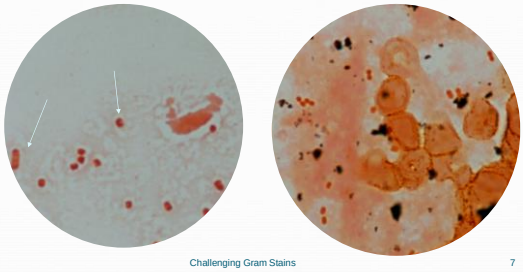
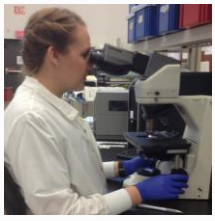
Enteric GNB – short

E coli *Acinetobacter*



GS Consistency Challenges:

- Variability
  - Non-standard specimen
  - Smear, stain quality
- Subjectivity
  - GS interpretation.
- CAP MIC.11350



Automated Stainers: One Solution for Consistent Stain Quality

CAP MIC.11350 GS Consistency

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

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CAP MIC.21530 GS Correlation

***REVISED** 07/31/2012*
MIC.21530 Direct Gram Stain Procedures **Phase I**

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)

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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 cultures clinically misleading without GS correlation.
- Appropriate monotherapy 94% of time when guided by GS.

Strand, CL., *Positive Blood Cultures, Can We Always Trust the Gram Stain?* Am J Clin Path 2006, 126:671-672.

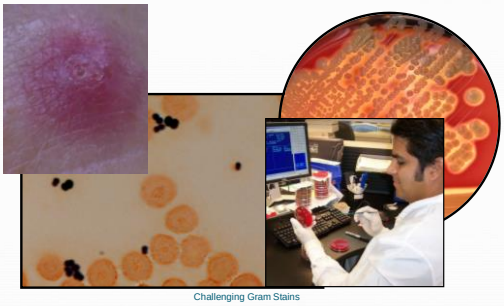
*Rand, Kenneth H, et al., *Errors in Interpretation of Gram Stains from Positive Blood Cultures*, AM J Clin Pathol 2006, 126:686-690.

Reed, W., Byrd, G., Gates R., et al, A Meta-Analysis of Sputum GS ,West J Med 1996; 165:197-204.

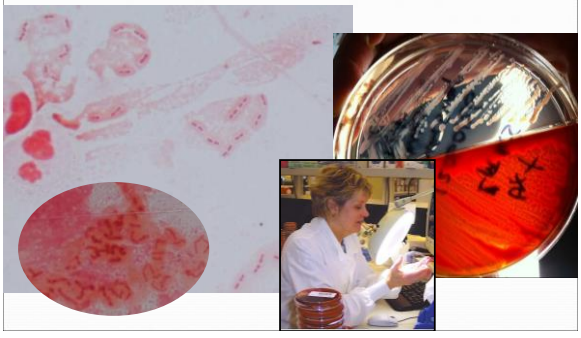
McCarter, Yvette, PhD, D(ABMM), ASM 2011 Clinical Core Curriculum III, Best Practices in the Work Up of Resp Cultures.

Challenging Gram Stains 1

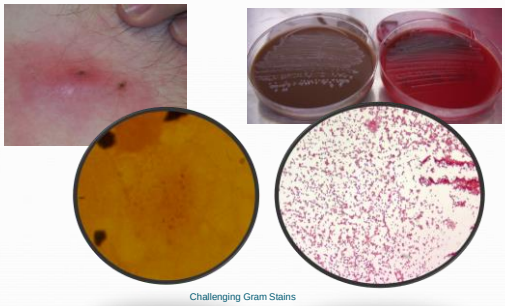
GS Correlation with Wound Culture



GS Correlation with Sputum Culture



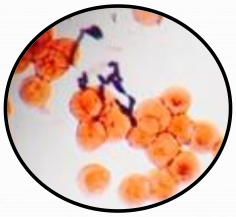
GS Correlation with Tickbite Culture

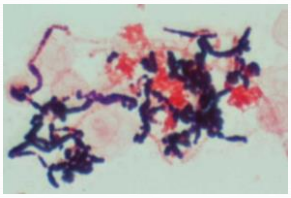


4th Case Challenge

NICU blood culture GS reported as budding yeast

Blood Culture= viridans Strep





GS Correlation QA

- Include in lab 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…

*Munson, E, Block T., et al, Mechanism to Assess GS Interp Prof of Techs at Satellite Labs, JCM Nov 2007, vol 45, no 11; 3754-3758. Dallas, Steven, Do Your GS Match Your Growth, Practical Response to CAP MIC.21530, ASM Clin Micro Portal Feb Hot Topic, 2014.

Local QA Plan: Improve GS Proficiency in Satellite Lab with Telemicroscopy

- Review each GS daily or in real time with core micro lab. Share expertise.
- Evaluate slide/stain quality and interpretation.
- Track correlation – accuracy rate.
- Monitor revised reports – provide feedback.
- Maximize GS results!

Telemicroscopy Success

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

Zuchowski, Linda, How Serious are you about Quality?, The Pathologist, Jan 2017, www.thepathologist.com

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.

*Rhoads, D., Sintchenko, V., Rauch, C., Clinical Microbiology Informatics, *Clin. Microbiol. Rev. October 2014 vol. 27 no. 4 1025-1047.*

McLaughlin WJ, Schifman RB, Ryan KJ, et al., Telemicrobiology: feasibility study. Telemed J 1998 Spring;4(1):11-7. Accessed 3-22-15 www.mcbi.nlm.nih.gov/pubmed/9599069.

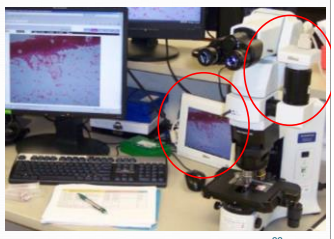
Telemicroscopy Advantages

- Enhance collaboration with health care partners.
- Public health consultation – share parasite images **www.cdc.gov/DPDx** (e.g.*Cyclospora* outbreak 2013).
- Bioterrorism preparedness (alternative to **STATPack™**)
- Boost QA program to meet CAP standards
- Allows satellite labs to keep blood cultures on-site.
- Utilize in any dept with microscopy.
- Contributes to positive patient outcome.
- Benefits entire health care system.

Campbell, Sheldon et al, The Clin Micro Lab in Diag of LRT Infections, JCM Vol 49, pS30-S33, Sept 2011.
Wolk, Donna, Dunne, Michael W. Jr., New Technologies in Clin Micro, JCM Vol 49, pS62-67, Sept 2011.

Telemicroscopy Equipment

- Microscope camera (Nikon DS-L2 on Olympus scope)
- Windows IP Configuration
- Controller unit
- Ethernet adaptor
- Local Area Connector
- No special software.
- Consult with vendors.



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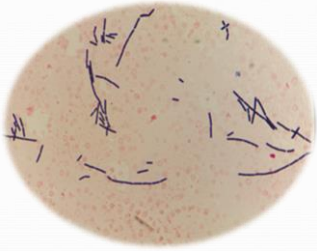
Smart Phone Camera Option

- Email digital images to experts for review



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Diagnosing BSI – Accurate GS

- 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, saves $.

Sogaard, Mette, et al., First Notification of Positive Blood Cultures and High Accuracy of the Gram Stain, JCM 4-2007, vol 45, no 4, 1113-1117, JCM.
Wolk, Donna, Dunne, Michael W. Jr., New Technologies in Clin Micro, JCM Vol 49, pS62-67, Sept 2011.
Uekahra Yuki, et al, Impact of Reporting GS Results from Blood Culture Bottles on Selection of Antimicrobial Agents, AJCP, 2009, 132, 18-25.

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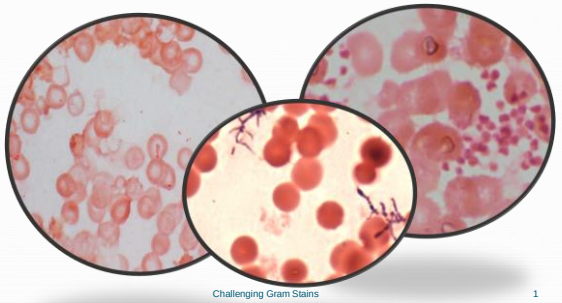
Blood Culture GS Diagnosis?



Diagnosing Bacterial Meningitis

- 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 - depending on pathogen and prior treatment.
  - Prior therapy decreases GS sensitivity to 40-60%.
- Spend extra time searching for pathogen if WBCs present – *H. flu* and *N. men* can be sparse!

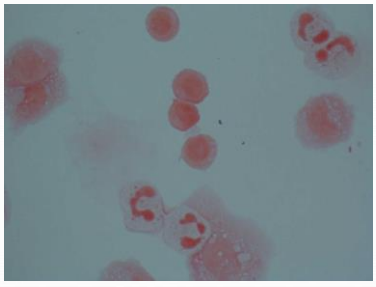
Baron E. et al., IDSA and ASM Guidelines 2013.
Brouwer M.C., et al., Epidemiology, Diagnosis, Antimicrobial Treatment of Acute Bacterial Meningitis, Clinical Micro Reviews, Vol 23, issue 3, July 2010. www.cdc.gov .
Tunkel, Hartman, Kaplan et al., CID 2004:39, 1 Nov. 1269-70.

CSF Gram Stain Study

- 2635 CSF specimens over 55 months
- 56 positive for bacterial or fungal meningitis
- 88% of gram stains show causative agent (48 of 56)
- 0.1% false positive (3 of 2635)
- Better morphology on cytospun specimens due to less G force in cytocentrifugation (8 min at 350g)

Dunbar et al., Microscopic Exam…of CSF, JCM, vol 35, p 1617-1620, 1998.

Cytospun Cell Morphology



CSF Gram Stain Tips

- Cytospin can increase sensitivity 100x ! (more sensitive than bacterial antigen test)
- Acridine orange stain helpful for intracellular bacteria (i.e. many PMNs and NOS)
- After 1 hour, 32% decrease in WBC detection
- 10-20% positive CSF Gram stains have neg cultures, but blood cultures pos 50-90%.

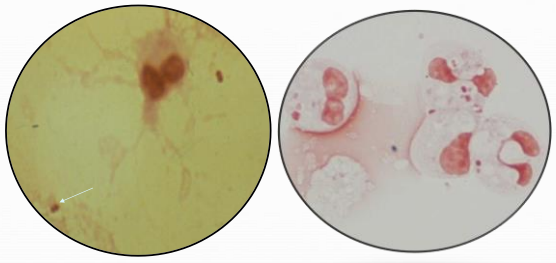
Baron, Ellen Jo, et al., IDSA and ASM Guidelines 2013.
Chapin-Robertson et al., Cytospin Increases Sensitivity… of CSF, JCM 30:377-380, 1992.
Farin Manian, MD, MPH, Detection and Treatment of CNS Infections, Chief Infect Diseases, Mercy Hospital, St.Louis,MO April 2012.
Karen Carroll, MD., "Bacterial Meningitis", ARUP Laboratory, Salt Lake City, March 1996.

CSF Sensitivity

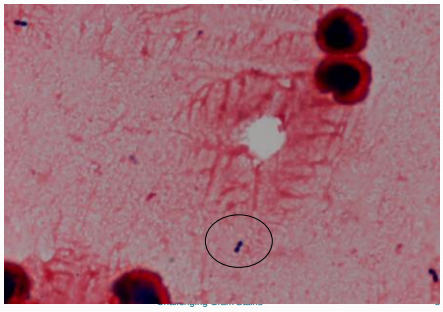
- Gram stain can have up to 93% sensitivity rate, prior to antibiotic therapy, depending on bacteria:
  - *Strep pneumo* 69-93% (most common, 61% of all cases)
  - *Strep agalactiae* 80-90%(66% cases in newborn 0-3 months)
  - *N.meningitidis* 30-89%
  - *Hemophilus influenzae* 25-65%
  - *Listeria* 23-36% (only 7% of cases, in elderly)
  - *Staph aureus* 20-44%
- Recognize age-related CSF pathogens, prevalence.

Brouwer M.C., et al., Epidemiology, Diagnosis, Antimicrobial Treatment of Acute Bacterial Meningitis, Clinical Micro Reviews, Vol 23, issue 3, July 2010.

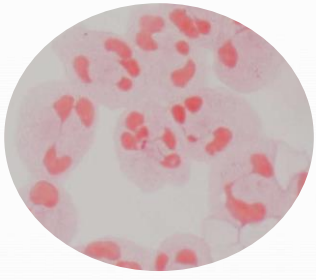
Neisseria meningitidis in CSF



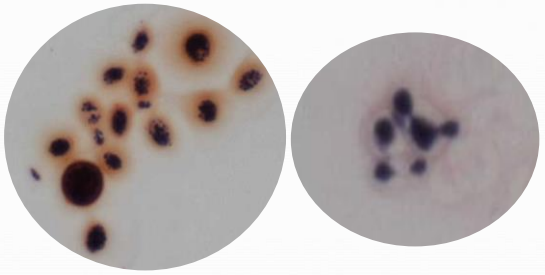
CSF with *Strep pneumo*



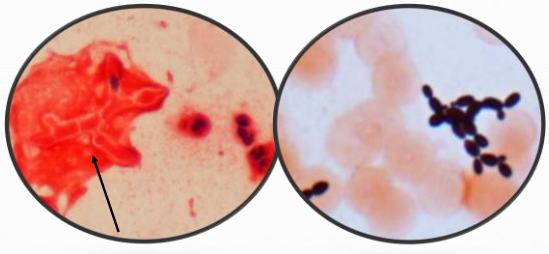
CSF with *E coli*



Cryptococcus in Blood and CSF, (non-compliant HIV+ patient)

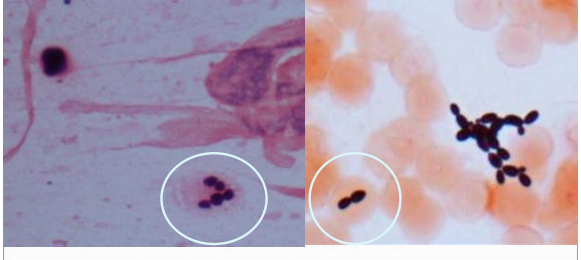


Diagnosing Fungal Infections – Are we Searching on Gram Stains?



Harrington, Brian J. et al., Gram Stains – Beyond the Basics, Lab Med, Vol 33, No 8, p609-614, Aug 2002.
Mohan, Subhash, Gram Stain - Looking Beyond Bacteria to Find Fungi in GS Smear, 2009.

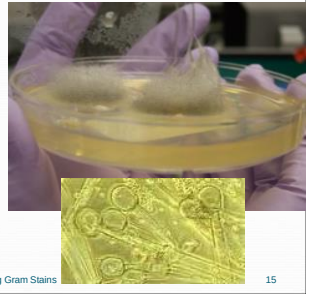
Budding Yeast or Artifact?



Septate Fungal Elements

Fatal Fungal Soft-Tissue Infections After a Tornado - Joplin, Missouri, 2011

- 1st known cluster assoc with tornado victims.
  - Lacerations, foreign body, blunt trauma, fractures.
- 13 confirmed patients yielded the Mucormycete *Apophysomyces trapeziformis*.
- Surgical debridement, culture positive.
- Cutaneous mucormycosis usually opportunistic in immuno-compromised with fatality rate of 29-83%.
- MMWR/CDC report: http://www.faqs.org/periodicals/201107/2413959571.html#ixzz1Umy2Oz2A



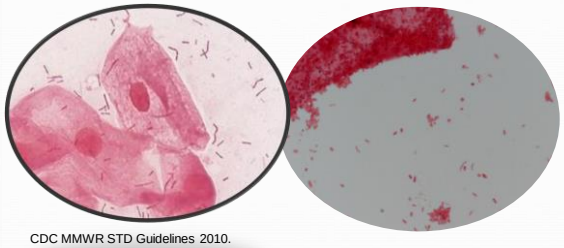
Diagnosing BV

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

MMWR CDC Sexually Transmitted Diseases Treatment Guidelines, 2010. ***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.
Bacterial Vaginosis: An Update on Dx and Rx: Expert Commentary and Five Year Review, www.MedScape.com
Carol Spiegel, Bacterial Vaginosis, Clinical Micro Review. 1991;4:485-502
Hogan VK et al, Relative performance of 3 methods for diagnosing BV, Maternal Child Health 2007.
Nugent, Krohn, Hillier, Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. JCM. 1991;29:297-301

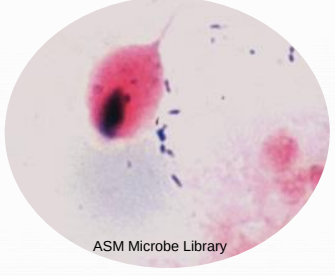
Graded Vaginal Gram Stain

Normal **Abnormal (BV)**



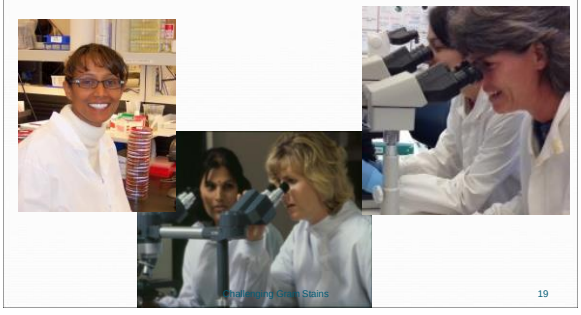
CDC MMWR STD Guidelines 2010.

Trichomonas on Vaginal Gram Stain
(confirm with acridine orange stain)



ASM Microbe Library

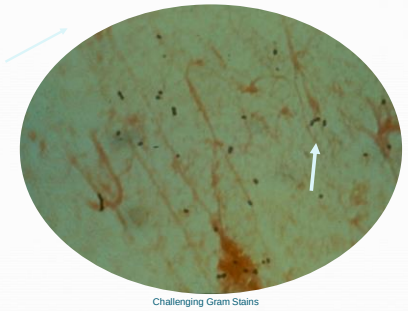
Gram Stain Case Studies



Case Study #1

- 62 year old with arm infection, called physician for antibiotic prescription
- Infection spread upwards to shoulder within several days
- Pt admitted for surgical intervention
- Stat Gram stain of shoulder tissue = *Staph*
- Surgeon suspects *Strep.* (No telemicroscopy.)
- Repeat GS = *Staph?*

Gram Positive Cocci Pairs



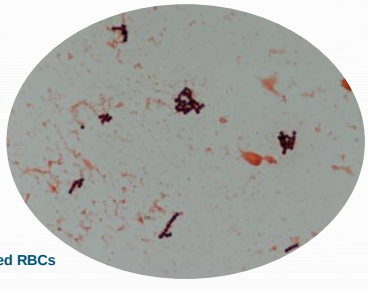
Case Study #1 (con't)

- Amputation of arm at shoulder
- Patient expired within 24 hours
- Shoulder tissue and blood cultures grew *Streptococcus pyogenes*
- Phage typing = "flesh eating" strain

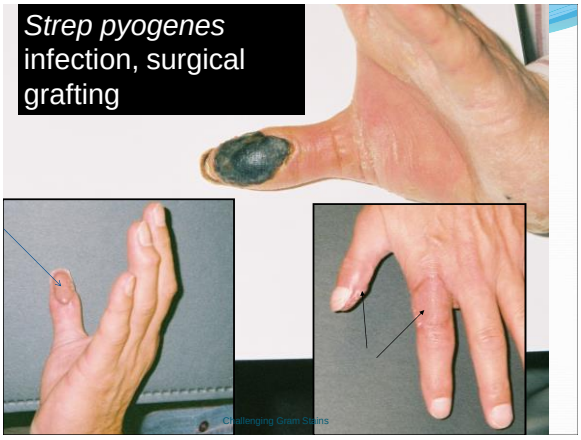
Case Study #2

- Healthy 45 year old male pricked thumb on his metal boot eyelet.
- Within 24 hours, acute thumb pain, low fever, red streak up arm. Doctor visit.
- Blood cultures drawn, oral antibiotics started.
- Admitted to hospital:
  - IV Ampicillin
  - surgical debridement of wound,
  - culture of drainage

GPC in Chains in Blood

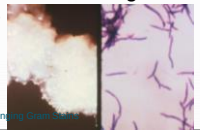


Note lysed RBCs

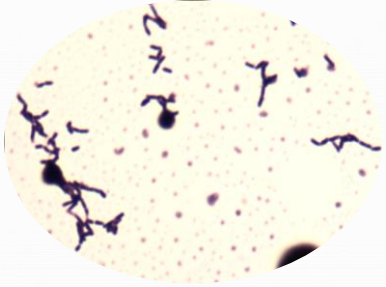


Case Study #3

- Post-menopausal woman with severe headache and flank pain, low grade fever
- CT scan = walnut size tumor or abscess
- Surgical drainage = purulent material
- Gram stain = short, branching Gram positive rods
- Partial acid-fast stain = negative
- 48 hours later, anaerobic culture grew tiny white molar tooth colonies



Branching Gram Pos Bacilli



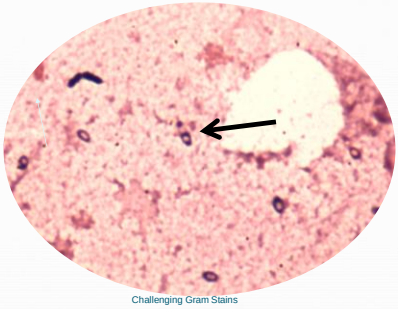
Case Study #3 (con't)

- Culture report = *Actinomyces israelli* and *Fusobacterium*
- GS review did not show any fusiform bacteria.
- Source of infection – patient admitted IUD still in place after 30 years!
- IUD removed and cultured, grew *Actino*
- Treatment = 3 week course penicillin
- Patient successfully recovered.

Case Study #4

- 44 year old truck driver, severe thigh pain
- History of leukemia, 1 year partial remission
- Examined in ER, dx = muscle cramp.
- Within 24 hrs, returned to ER, X-ray = gas in tissue = probable cellulitis, gas gangrene
- Surgery sent thigh tissue for stat Gram stain
- GS report = Gram negative rods
- Surgeon questioned result (no telemicroscopy)
- Repeat GS = Gram positive rods?

Large GPB with Spores in Blood



Anaerobic Swarming Growth

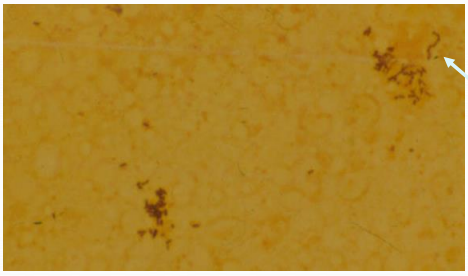
Case Study #4 (con't)

- Leg amputated
- Patient expired < 24 hours post admission
- Thigh tissue and blood cultures grew *Clostridium septicum* (anaerobic swarmer)
- Pathogen in patients with hematologic disorders, endogenous origin.

Case Study #5

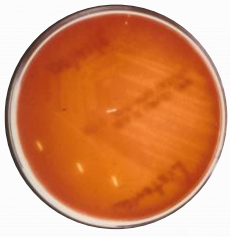
- 77 year old diabetic, former cancer patient, admitted with FUO, altered mental status
- WBC = 9,200 26% bands
- X-rays, ultrasound, CT scan negative
- R/o UTI, pneumonia, meningitis
- Empiric treatment = Rocephin
- Blood culture GS = small gprs and *Strep*

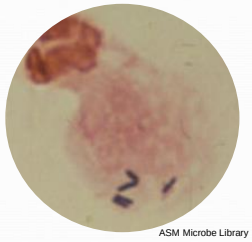
Blood with GPCB, GPC in Chains? Lysed RBCs



Morphology

Beta hemolysis on blood agar **Intracellular GPB**





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Case Study #5 (con't)

- Blood cultures grew *Listeria monocytogenes*
- *Listeria* can appear as GPCB which can chain up.
- Discussion of recent lunch meat recall prompts patient's wife to bring meat samples to lab
- *Listeria* also isolated in pure culture from meat
- Health dept notified, USDA collects samples
- CDC confirmed different strain, different manufacturer
- New official, international recall of meat
- Patient recovered despite 25% mortality rate.

Summary: How can we Meet Gram Stain Challenges?

- **Identify challenges in lab settings**
  - Bridge gap between core lab and satellites, non-micro techs.
  - Partner, communicate, provide support, share expertise.
- **Implement quality improvement processes:**
  - Monitor GS accuracy rate (meet CAP requirements)
  - Improve GS expertise with robust training, feedback
    - Optimize specimen and stain quality
    - Target pathogen, clinically relevant results
  - Utilize technology – telemicroscopy
- **Maximize positive patient outcomes**

New Gram Stain Atlas

- Gram Stain Benchtop Reference Guide, D. Jane Hata, Richard B. Thomson Jr, illustrated www.ebooksCAP.org, 2017.

Thank you!

Questions?

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