Gram’s Stain
for
Primary Care Physicians

Toru Kamiya, MD
Resident at UHIMRP
2002-2005
Background

• In the past, physicians typically performed Gram’s stains by themselves on body fluids and other clinically relevant materials from patients with suspected infections to assist in diagnosis and management.

• Although ACGME requires competence in interpretation of Gram’s stain, currently residents do not routinely perform Gram’s stains by themselves.

• Often clinical decisions about diagnosis and antibiotics are made without adequate consideration of Gram’s stain data.
In 1988, the Clinical Laboratory Improvement Amendments (CLIA) established quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patients test results.

However, the difficulty in complying with sometimes cumbersome CLIA requirements resulted in fewer Gram’s stains by independent practitioners and residents. In addition, the move of the DLS micro lab off site from QMC made Gram’s stains review less accessible to residents.

Furthermore, limited evidence about the utility of Gram’s stain have made the test away from the frontline tools for primary care physicians in the era of EBM.
Background

- In fact, **ATS guideline of community acquired pneumonia** does not recommend Gram’s stain as a routine initial diagnostic testing. It states that “If a drug-resistant pathogen, or an organism not covered by usual empiric therapy is suspected, sputum culture should be obtained, and **Gram’s stain** should be used to **guide interpretation of culture results**. In general, **sputum Gram’s stain cannot be used to focus initial empiric antibiotic therapy**, but could be used to broaden initial antibiotic therapy to include organisms found on the Gram’s stain that are not covered by the usual initial empiric antibiotic therapy options.”
Background

- As a primary care provider, I have performed Gram’s stains by myself and have actively utilized the test for diagnoses and treatments of my pts for more than 10 years in Japan. I believe that Gram’s stain is still a useful, interesting, fun tool in the management of infectious diseases.

- I hope these slides help residents understand the usefulness and limitations about Gram’s stain, and begin to routinely use them in the management of pts with infections.
Module goals

1) Resident will learn the **patterns** of Gram’s Stain (GS) for **clinically important bacteria**.

2) Resident will **interpret** and **utilize** the result of GS reports.

3) Resident will learn the **situations** when the information of GS is helpful.
Gram’s Stain preparation

- **GRAM +**
  - Fixation
  - Crystal Violet
  - Iodine treatment
  - Decolorization
  - Counter stain (safranin)

- **GRAM -**
GS categorizes bacteria into 4 groups.

<table>
<thead>
<tr>
<th></th>
<th>Gram positive</th>
<th>Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocci</td>
<td><img src="image" alt="Black Cocci" /></td>
<td><img src="image" alt="Pink Cocci" /></td>
</tr>
<tr>
<td>Rods</td>
<td><img src="image" alt="Black Rods" /></td>
<td><img src="image" alt="Pink Rods" /></td>
</tr>
</tbody>
</table>
Patterns of GS for clinically important bacteria

Gram Positive Cocci (GPC)

<table>
<thead>
<tr>
<th>In clusters</th>
<th>Staphylococci</th>
<th>Staphylococcus aureus (MSSA, MRSA)</th>
<th>Coagulase negative staph.</th>
<th>Staphylococcus saprophyticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>In pairs and chains</td>
<td>Streptococci</td>
<td>Strep. pneumoniae</td>
<td>Strep. pyogenes</td>
<td>Strep. viridans</td>
</tr>
</tbody>
</table>

* Staph sp. is sometimes mistakenly read as “GPC in pairs and chains”.
Patterns of GS for clinically important bacteria

<table>
<thead>
<tr>
<th>Gram Positive Rods (GPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Corynebacterium diphtheria</em></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td><em>Clostridium tetani</em></td>
</tr>
<tr>
<td><em>Bacillus anthracis</em></td>
</tr>
</tbody>
</table>
Patterns of GS for clinically important bacteria

<table>
<thead>
<tr>
<th>Gram Negative Cocci (GNC)</th>
<th>Neisseria meningitidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neisseria sp.</td>
<td>Neisseria gonorrhoeae</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
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</tr>
</tbody>
</table>

“kidney-bean-shaped”

*May also be called Gram-negative Diplococci “GNDC”.*
Patterns of GS for clinically important bacteria

<table>
<thead>
<tr>
<th>Gram Negative Rods (GNR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
</tr>
<tr>
<td><strong>Proteus sp.</strong></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
</tr>
<tr>
<td><strong>Enterobacter sp.</strong></td>
</tr>
<tr>
<td><strong>Citrobacter sp.</strong></td>
</tr>
<tr>
<td><strong>Acinetobacter sp.</strong></td>
</tr>
<tr>
<td><strong>Stenotrophomonas maltophilia</strong></td>
</tr>
<tr>
<td><strong>Bacteroides sp.</strong></td>
</tr>
</tbody>
</table>

| **Haemophilus influenzae** |

* *Haemophilus influenzae* is sometimes categorized in *Gram-negative coccobicili.*
<table>
<thead>
<tr>
<th>Lactose fermenter</th>
<th>Non-lactose fermenter</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eschericia coli</em></td>
<td><em>Pseudomonas sp.</em></td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td><em>Proteus mirabilis</em></td>
</tr>
<tr>
<td><em>Citrobacter sp.</em></td>
<td><em>Serratia marcescens</em></td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td><em>Morganella morganii</em></td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td><em>Providencia sp.</em></td>
</tr>
</tbody>
</table>

You will find it helpful to remember above yellow and pink organisms when you see the reports of GS. They are sometimes described as “GNR lactose fermenter” or “GNR non-lactose fermenter”.

< Enterobacteriaceae >
General rules about GS

- GS will identify general categories of bacterial species, e.g. *Staphylococcus species*, *Streptococcus species*.

- However, one can focus on more specific organisms when GS information is combined with knowledge of common etiologic pathogens for a particular clinical infection.

- For example, when the result of sputum GS for pts with community acquired pneumonia shows **GNR**, we need to consider *Klebsiella pneumoniae*. On the other hand, if that’s the case with pts with nosocomial pneumonia, we need to consider *Pseudomonas aeruginosa*. 
Let’s learn about **Sputum GS**!

- For sputum GS, check the number of *epithelial cells* and WBC to assess the quality of the specimens before looking at the bacterial report.

**Inadequate sputum sample for culture**

- *epithelial cells* > 2+ or > 25 / LPF

* LPF: low power field (x10)

- The greater the number of epithelial cells, the greater likelihood of *contamination by saliva*.

**Ideal sputum sample for culture**

- *epithelial cells* ≤1+ or < 10 / LPF
- WBC > 1+ or > 10 / LPF
• Varied types of bacteria in great numbers are often found near epithelial cells. These almost always are organisms that constitute the normal flora of the contaminating cell source.
Case studies

• Followings are clinical scenarios with high quality GS photos.

• These photos were taken under HPF (high power field: x1000) magnification.
65 yo male w/ COPD was admitted for pneumonia.

Sputum GS report shows

**GPC, in pairs and chains**

Which organism do you suspect?

**Answer:** *Streptococcus sp.*

(especially, *Streptococcus pneumoniae* in sputum)
65 yo male w/ COPD was admitted for pneumonia.

Sputum GS report shows GNR, lactose fermenter

Which organism do you suspect?
Answer: Klebsiella pneumoniae
65 yo male w/ COPD was admitted for pneumonia.

Sputum GS report shows

**Gram negative cocci** or Gram negative diplococci

Which organism do you suspect?

Answer: *Moraxella catarrhalis*
65 yo male w/ COPD was admitted for pneumonia.

Sputum GS report shows

**Gram negative rods** or Gram negative coccobacilli

Which organism do you suspect?

Answer: *Haemophilus influenzae*
65 yo male w/ COPD was admitted for respiratory failure. He was intubated and put on a ventilator. On the 7th hospital day, he developed fever and new infiltrates.

Sputum GS report shows **GPC, in clusters** Polymorphonuclear WBC

Which organism do you suspect?
Answer: *Staphylococcus aureus* (MRSA or MSSA)

* Staph sp. is sometimes mistakenly read as “GPC in pairs and chains”.

"clusters"
65 yo male, nursing home resident w/ CVA, recurrent pneumonia was admitted for pneumonia.

Sputum GS report shows

**GNR**

Which organisms do you suspect?

Answer: *Pseudomonas aeruginosa, E. coli, Stenotrophomonas m., Acinetobacter c., Enterobacter sp.*

Nosocomial organisms
45 yo Filipino male presented with chronic cough. CXR showed infiltrates at LUL.

Sputum GS repeatedly shows Normal throat flora

Which organism do you need to consider?

Answer: Mycobacterium Tuberculosis
Clinical tips for Urine GS!

• As a general rule, 75-90 % of uncomplicated UTI is due to *Escherichia coli*.

• Therefore, most of the Urine GS reports come back as “GNR”.

• However, several % of uncomplicated UTI is caused by *Enterococcus species* which are not susceptible to all the cephalosporin antibiotics.

• When you see “GPC in pairs and chains” in urine GS report, you need to assume *Enterococcus species* and to consider using ampicillin or vancomycin, instead of using cephalosporins.

* When you see ≥ 1 organism / HPF in unspun urine, you can assume that UCx will grow ≥ 100,000 cfu / ml.
35 yo female presented with uncomplicated UTI.

Urine GS report shows

**GNR, lactose fermenter**

Which organism do you suspect?

**Answer:** *Escherichia coli*
65 yo male w/ BPH presented with UTI.

Urine GS report shows

**GPC, in pairs and chains**

Which organism do you suspect?

**Answer:**  *Enterococcus species*  

Consider ampicillin or vancomycin  
All cephalosporins ineffective
73 yo female, nursing home resident w/ CVA, recurrent UTI, chronic indwelling Foley cath, was admitted for complicated UTI.

Urine GS report shows **GNR, lactose non-fermenter**

Which organisms do you suspect?

Answer: *Pseudomonas aeruginosa*, or other nosocomial pathogens
63 yo female was admitted to ICU for PNA. Pt required mechanical ventilation and was given broad spectrum ABx for 2wks. She developed new fever.

Urine GS report shows **Yeast**

Which organism do you suspect?

Answer: *Candida sp.*
25 yo male h/o STD presented with penile discharge.

GS of urethral discharge report shows GNC or GNDC

phagocytosis by polymorphonuclear WBC

Which organism do you suspect?

Answer: *Neisseria gonorrhoeae*

* The presence of phagocytosis generally suggests true infection rather than colonization.
58 yo male h/o ESRD on HD was admitted for fever. 2wks ago, pt was placed vas cath at R IJ for the occlusion of AV fistula.

GS of blood culture report shows **GPC, in clusters**

Which organisms do you suspect?

Answer: **Staphylococcus sp. (MRSA vs MSSA vs coag. neg. staph.)**

consider covering **MRSA** and removing vas cath

* Staph sp. is sometimes mistakenly read as “GPC in pairs and chains”.
58 yo male h/o rheumatic heart dz. was admitted for fever. Echocardiogram showed vegetations on mitral valves.

GS of blood culture report shows

**GPC, in pairs and chain**

Which organism do you suspect?
Answer: *Streptococcus sp.*
Bacterial meningitis

• Acute bacterial meningitis is a medical emergency.

• The most important initial issues are:
  1) rapid diagnosis
  2) early and appropriate ABx

• Ideal sequence of management
  BCx + Dexamethasone → empiric ABx → LP in 30 min

• If focal neurological deficit, seizure, GCS≤12,
  BCx + Dexamethasone 1) → empiric ABx → CT → LP

  1) Dexamethasone 0.15mg/kg IV q6h for 4 days
Bacterial meningitis

- GS of CSF is rapid, inexpensive, highly specific (> 97%) for the diagnosis of bacterial meningitis.

- If the GS is highly suggestive, ABx should be targeted toward the presumed pathogen.

- ABx should be modified once the CSF Cx and susceptibility results available.
3 categories in acute bacterial meningitis

- Empiric Therapy – CSF Gram stain is negative
- Empiric Therapy – Positive CSF Gram stain
- Specific Therapy
### Acute bacterial meningitis

**Empiric Therapy - Positive CSF Gram stain**

<table>
<thead>
<tr>
<th>Group</th>
<th>Organism</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPDC</td>
<td><em>S. pneumoniae</em></td>
<td>Ceftriaxone or cefotaxime + vancomycin</td>
</tr>
<tr>
<td>GPR</td>
<td><em>Listeria</em></td>
<td>AMP + gentamicin</td>
</tr>
<tr>
<td>GNDC</td>
<td><em>N. meningitidis</em></td>
<td>Pen G</td>
</tr>
<tr>
<td>GNR</td>
<td><em>H. influenzae</em></td>
<td>Ceftazidime + gentamicin</td>
</tr>
<tr>
<td></td>
<td>coliforms</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em></td>
<td></td>
</tr>
</tbody>
</table>
Please chose one right answer.

Question 1) – 20)
1) Sputum GS

- Which is the finding of *inadequate sputum* sample for culture?

  A) WBC $\geq 1+$ (>10 / LPF)

  B) epithelial cells $\geq 2+$ (> 25 / LPF)

  C) epithelial cells $\leq 1+$ (<10 / LPF)
2) 65 yo male w/ COPD was admitted for pneumonia.

Sputum GS report shows **GPC, in pairs and chains**

Which organism do you suspect?

A) *Streptococcus pneumoniae*
B) *Moraxella catarrhalis*
C) *Haemophilus influenzae*
3) 65 yo male w/ COPD was admitted for pneumonia.

Sputum GS report shows

**GNR, lactose fermenter**

Which organism do you suspect?

A) *Pseudomonas aeruginosa*
B) *Klebsiella pneumoniae*
C) *Haemophilus influenzae*
4) 65 yo male w/ COPD was admitted for pneumonia. Sputum GS report shows **Gram negative cocci** or Gram negative diplococci

Which organism do you suspect?

A) *Streptococcus sp.*
B) *Moraxella catarrhalis*
C) *Haemophilus influenzae*
5) 65 yo male w/ COPD was admitted for pneumonia. Sputum GS report shows **Gram negative rods** or Gram negative coccobacilli

Which organism do you suspect?

A) *Streptococcus* sp.
B) *Moraxella catarrhalis*
C) *Haemophilus influenzae*
6) 65 yo male w/ COPD was admitted for respiratory failure. He was intubated and put on a ventilator. On the 7th hospital day, he developed fever and new infiltrates. Sputum GS report shows **GPC, in clusters**

Which organism do you suspect?

A) *Streptococcus* sp.
B) *Corynebacterium* sp.
C) *Staphylococcus aureus*
7) 82 yo female, nursing home resident w/ h/o recurrent pneumonia was admitted for pneumonia.

Sputum GS report shows **Gram negative rods**

Which organism do you suspect?

A) *Escherichia coli*

B) *Pseudomonas sp.*

C) *Moraxella catarrhalis*
8) 45 yo Filipino male presented with chronic cough. CXR showed infiltrates at LUL.

Sputum GS repeatedly shows

**Normal throat flora**

Which organisms do you need to consider?

A) *Staphylococcus aureus*
B) *Mycobacterium Tuberculosis*
C) *Pseudomonas sp.*
D) *Cryptococcus sp.*
9) 23 yo healthy female was admitted for UTI.

Urine GS report shows

**GNR, lactose fermenter**

Which organism do you suspect?

A) *Escherichia coli*
B) *Pseudomonas sp.*
C) *Moraxella catarrhalis*
10) 65 yo male w/ BPH was admitted for UTI.

Urine GS report shows **GPC, in pairs and chains**

Which organism do you suspect?

A) *Staphylococcus* sp.
B) *Corynebacterium* sp.
C) *Enterococcus* sp.
11) Continued from question 10)

Urine GS report shows **GPC, in pairs and chains**

Which antibiotics do you choose?

A) Ceftriaxon
B) Ampicillin or vancomycin
C) Cefazolin
12) 73 yo female, nursing home resident w/ CVA, recurrent UTI, chronic indwelling Foley cath, was admitted for complicated UTI.

Urine GS report shows **GNR, lactose non-fermenter**

Which organism do you suspect?

A) *Moraxella catarrhalis*
B) *Escherichia coli*
C) *Pseudomonas sp.*
13) 63 yo female was admitted to ICU for respiratory failure d/t PNA. Pt was given broad spectrum ABx for 1wk.

Urine GS report shows yeast

Which organism do you suspect?

A) Candida sp.
B) Cryptococcus sp.
C) Aspergillus sp.
14) 24 yo male h/o STD presented w/ penile discharge.

GS of urethral discharge report shows **Gram negative cocci** or Gram negative diplococci

Which organism do you suspect?

A) *Moraxella catarrhalis*
B) *Neisseria meningitidis*
C) *Neisseria gonorrhoeae*
15) Continued from question 14)

Gram’s stain of urethral discharge report shows **Gram negative cocci**

Bacterial phagocytosis by WBC usually suggests

A) *Colonization*
B) *Contamination*
C) *True infection*
16) Chose the right combination

A) Lactose fermenter : *Pseudomonas sp.*

B) Lactose fermenter : *Eschericia coli*

C) Lactose non-fermenter : *Klebsiella sp.*
17) 58yo male w/ ESRD on HD was admitted for fever. He was placed a vas cath to R IJ 3 wks ago for AV fistula occlusion.

GS of blood culture shows **GPC, in clusters**

Which organisms do you suspect?

A) Coagulase-negative staphylococci
B) Staphylococcus aureus (MSSA)
C) Staphylococcus aureus (MRSA)
D) All of the above
18) Choose the correct statement(s)

A) *Staphylococcus sp.* is sometimes mistakenly read as Gram positive cocci in pairs and chains.

B) Gram positive rods in CSF suggests *Listeria monocytogenes*.

C) *Campylobacter sp.* can be recognized in stool Gram’s stain.

D) All of the above
19) 58 yo male h/o rheumatic heart dz. was admitted for fever. Echocardiogram showed vegetations on mitral valves.

GS of blood culture
GPC, in pairs and chains

Which organism do you suspect?

A) *Staphylococcus* sp.
B) *Corynebacterium* sp.
C) *Streptococcus* sp.
20) Choose the correct statement(s)

A) GS of CSF is rapid, highly specific (> 97%) for the diagnosis of bacterial meningitis.
B) If the GS is highly suggestive, ABx should be targeted toward the presumed pathogen.
C) The most important initial issues in bacterial meningitis are rapid diagnosis and early, appropriate ABx administration.
D) All of the above
Welcome to the wonderful world of Gram’s stains!

From next slide, you will see variety of situations where GSs are helpful. Have fun!
68 yo female w/ steroid dependent asthma was admitted for pneumonia. Pt didn't respond to empiric ABx for CAP.

Sputum GS shows

**gram-positive branching, beaded filaments**

Which organisms do you suspect?

Answer: *Nocardia sp.*  
Rx: TMP/SMX
67 yo female w/ AML, s/p chemo, neutropenic fever for 2 wks developed new patchy infiltrates in CXR.

Sputum GS shows

**gram-positive, acute branching septate hyphae**

Which organisms do you suspect?

Answer: *Aspergillus sp.*

Consider invasive aspergillosis
15 yo previously healthy male was admitted for acute gastroenteritis.

Stool GS shows **gram-negative rods, small curved, “gull wing”**

Which organisms do you suspect?

Answer: *Campylobacter sp.*

*Campylobacter sp.* is the only organism which can be recognized by stool Gram’s stain.
52 yo post officer presented with shock and respiratory failure.

CXR

- widened mediastinum

CT of chest

- mediastinum lymphadenopathies
- diffuse infiltrates

GS of blood culture

Which organisms do you suspect?

Answer: *Bacillus anthracis*
Inhalational Anthrax

*CDC*
52 yo sheep farmer presented with painless black skin lesion.

Wound GS shows

gram-positive, spore-forming rods

Which organisms do you suspect?

Answer: *Bacillus anthracis*, “cutaneous Anthrax” *CDC*
82 yo female w/ CVA was admitted for aspiration pneumonia.

Sputum GS typically shows **polymicrobial pattern**

We can see polymicrobial organisms with phagocytoses by WBCs. This is a typical pattern for aspiration pneumonia.
57 yo male with s/p renal transplant presented with pneumonia.

Sputum GS report shows **Gram positive rods**

Which organisms do you suspect?

**Answer:** *Corynebacterium sp.*
85 yo male was admitted for fever and headache.

CSF GS shows

**Gram positive rods**

Which organisms do you suspect?

Answer: *Listeria monocytogenes*
CSF GS

Gram-positive cocci

CSF culture: *MRSA*
Wound GS

Gram-positive cocci, in pairs and chains

*Streptococcus pyogenes*

Gram-negative rods

*Bacteroides fragilis*
Wound GS

Gram-positive rods, spore forming

*Clostridium tetani*

Gram-negative coccobacilli

*Pasteurella multocida*
Ascites GS

Gram-positive cocci, in pairs and chains

Enterococcus faecium
Vaginal secretion

Gram-positive rods

*Lactobacillus sp.* (normal flora)
Cryptococcus neoformans

Yeast

With reddish halos and budding

India ink stains

Yeast

CSF
Acknowledgments

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• I’m also grateful to Dr. Erlaine F. Bello for her invaluable comments and suggestions as we proceeded.

Reference

• “Sputum Gram Stain Grading Criteria”
• Loyola University Health System, Microbiology & Immunology, Gram Stain
  http://www.meddean.luc.edu/lumen/DeptWebs/microbio/med/gram/gram-stn.htm