Yes, I'm Sure That Is What I Saw on the Gram Stain

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OUTLINE

I. Quality of slides

II. Sputum screen

III. Positive blood cultures

IV. Lower reproductive tract specimens

V. Potpourri

VI. Your questions (at any time; please go thru Erin)

The presenters state no conflict of interest and have no financial relationship to disclose relevant to the content of this presentation.

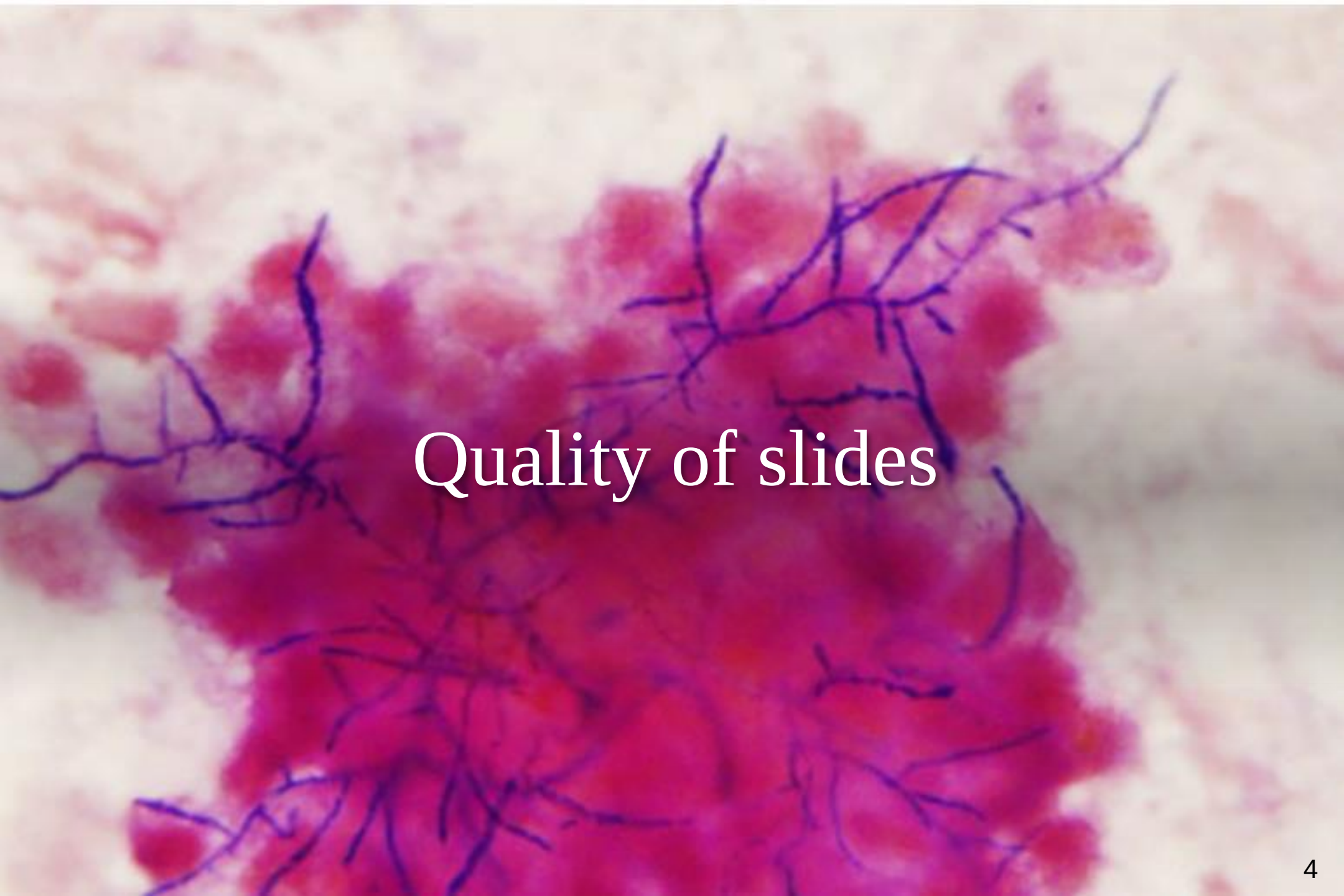
HERE'S HOW IT WILL WORK

A. Couple of introductory thoughts per topic

B. Drive around a few slides

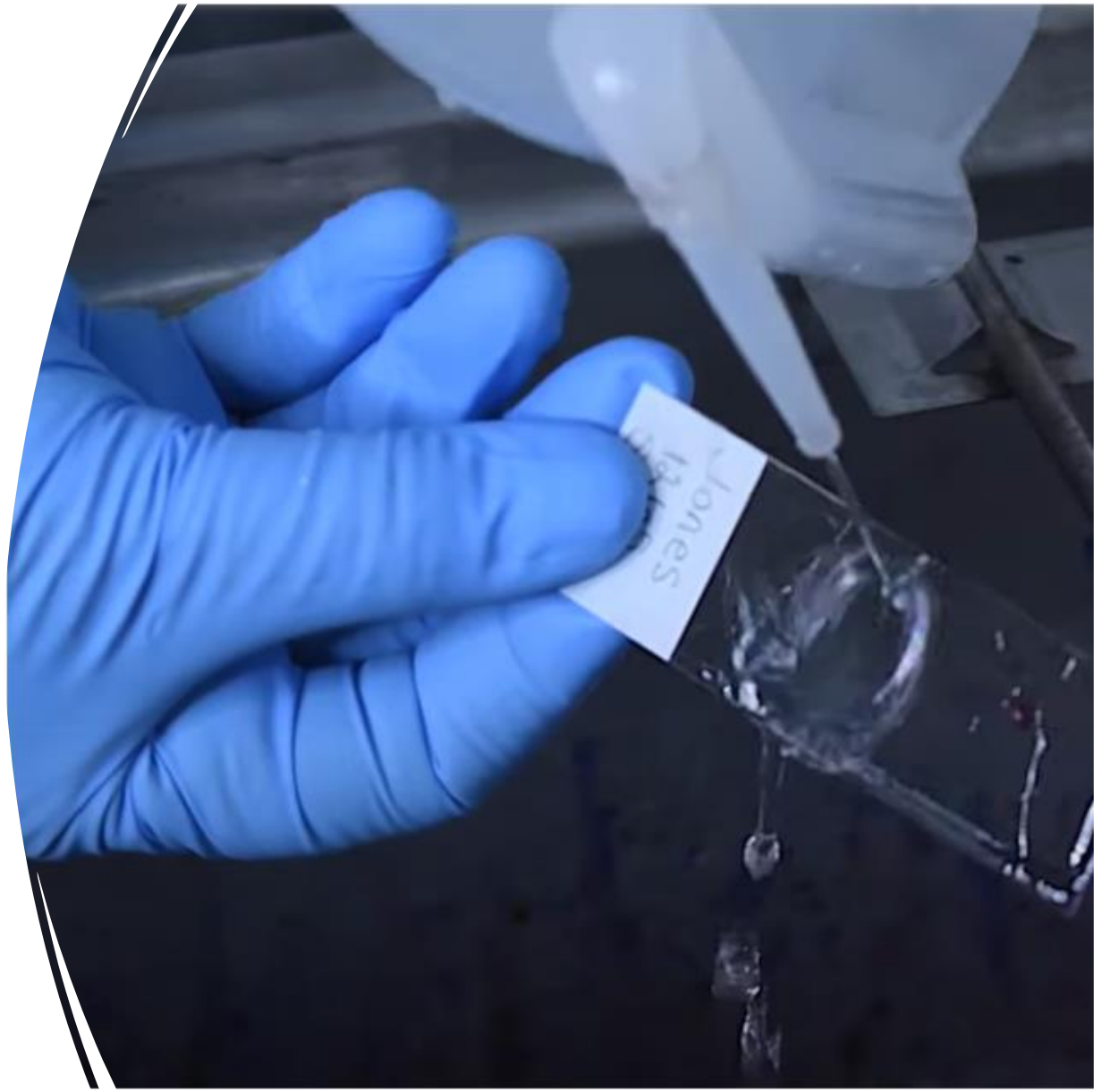
C. Your questions (at any time; please go thru Erin)

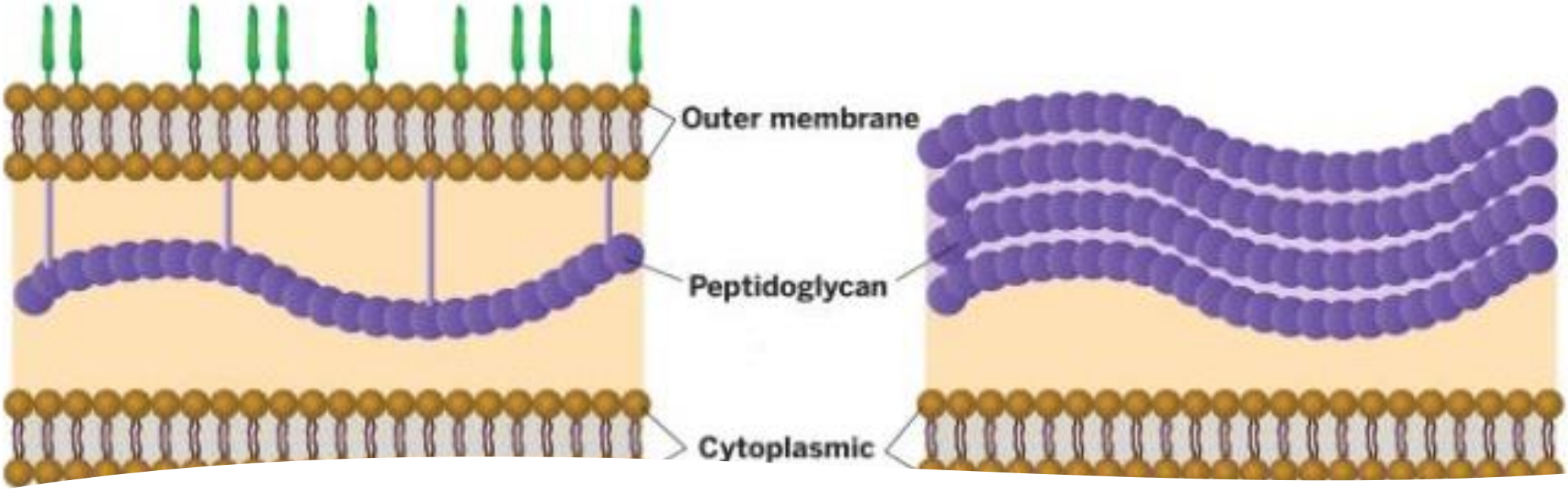
Limitations: Pregnant pauses
We may not have time for everything


Quality of slides

Decolorization

- Proper decolorization is essential to a good Gram stain
- The slide should be held at about a 45-degree angle
- The entire surface of the slide should be washed with decolorizer until blue/purple stops emitting from the **majority** of the slide.
- Immediately rinse with water to avoid accidentally overdecolorizing

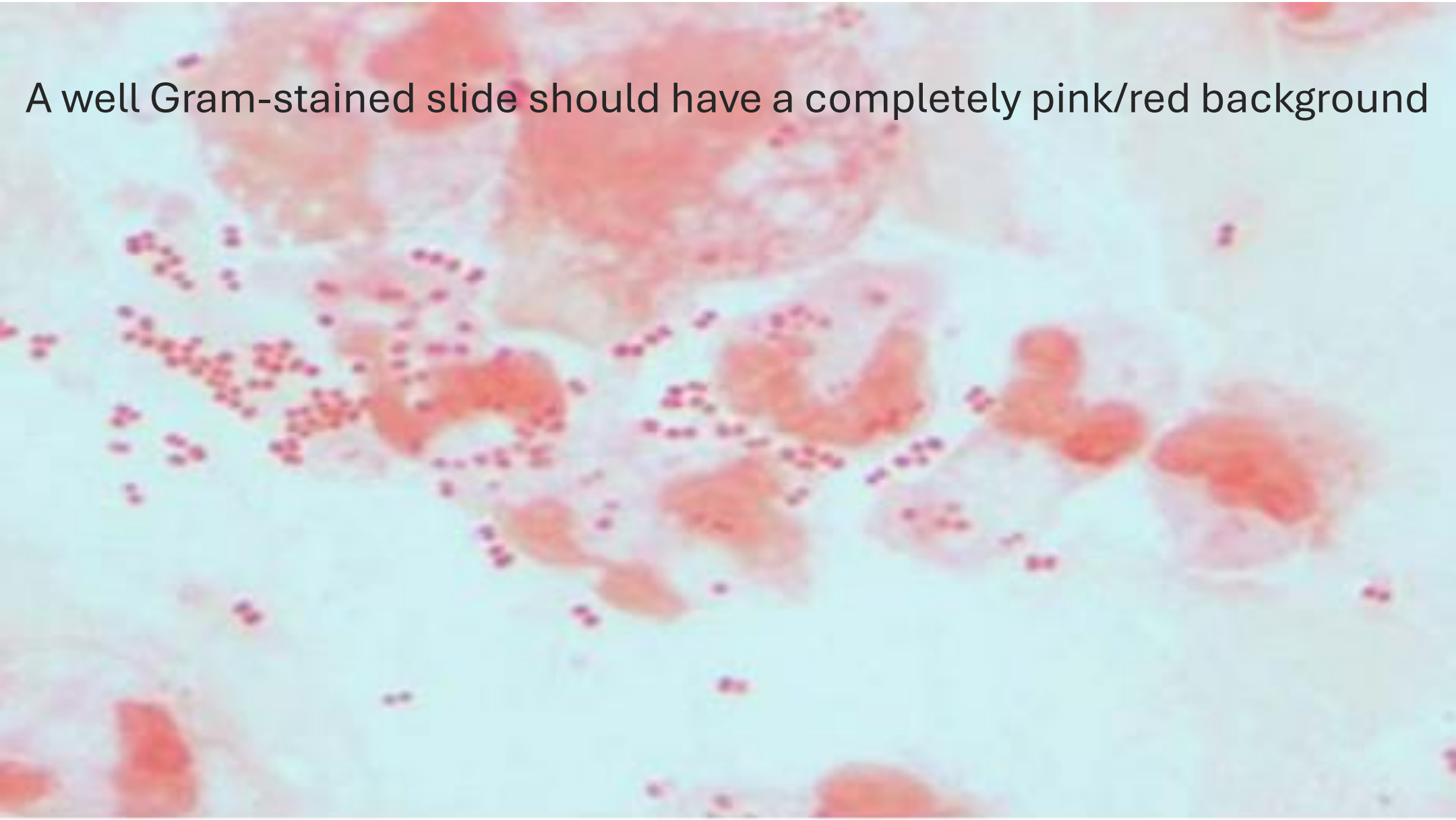




The amount of peptidoglycan in an organism's cell wall typically determines how much crystal violet is retained by the organism

- Gram-positive organisms have more peptidoglycan (50-90%) in their cell walls than Gram-negative organisms (around 10%) and thus have a propensity to retain the purple crystal violet/iodine stain complex better.

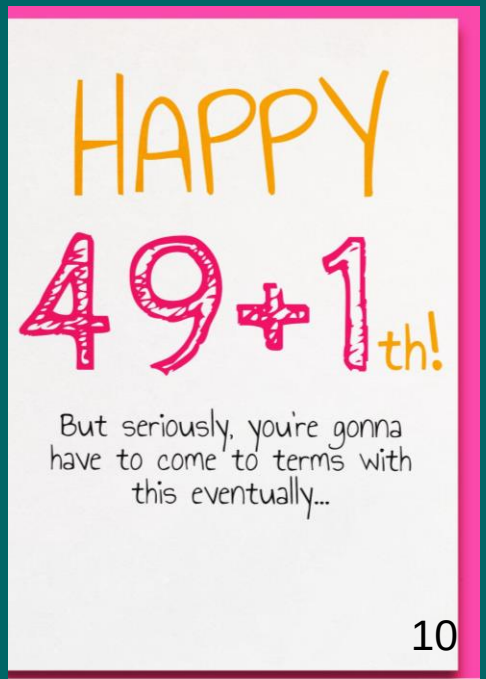
A well Gram-stained slide should have a completely pink/red background



Poorly Gram-stained slides will have blue/purple backgrounds

Scope time

Sputum screen



PRIMARY SPUTUM SCREENING

Epithelial cells Per LPF	PMN per LPF	Specimens	Mean species isolated (no.)
> 25	< 10	54	4.2
< 10	> 25	99	2.7

Mayo Clin Proc. 50:339-344; 1975

PREDOMINANT BACTERIA

Organism	"Rejected" Sputa	"Acceptable" Sputa
Viridans *Streptococcus*	60	31
Staphylcoccus epidermidis	13	3
Neisseria species	13	7
Haemophilus parainfluenzae	4	2
Yeast	4	2
Corynebacterium species	2	0
Haemophilus influenzae	0	8
Moraxella species	0	1
Streptococcus pneumoniae	0	4
Staphylococcus aureus	0	6
Klebsiella species	0	12
Other enterics	2	12
Pseudomonas aeruginosa	2	12

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CMPH COMMENTARY

- Actionable data best when numerous inflammatory cells plus:

  Gram-positive diplococci (57% sensitivity)
  Gram-negative coccobacilli (92% sensitivity)

- Most important role lies in specimen acceptability

- Sputum screen not applicable to:

  Legionella spp.
  Nocardia spp.
  Acid-fast bacilli
  Specimens from cystic fibrosis

Clinical Microbiology Procedures Handbook 5th ed. (volume 1)

CMPH RECOMMENDATIONS

- Select purulent or blood-tinged portions of pus or sputum with sterile “device”
- Try to produce thin film (caveats); dilute in drop of sterile saline (or press with a second slide)
- Examine 20-40 fields of view (10X objective)

  Reject when mean of ≥ 10 squamous epithelial cells

  When polymorphonuclear leukocytes are 10X more than squamous epithelial cells, accept if 3+ or 4+ of a single bacterial morphotype

Clinical Microbiology Procedures Handbook 5th ed. (volume 1)

Scope time

Positive blood cultures

Blood cultures

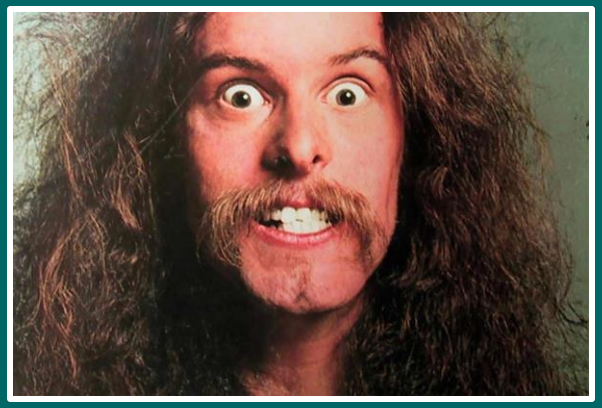
Positive blood culture Gram stains can have significant impact on how a patient is treated based on-

- How long it takes for the bottle to go positive in the instrument
- The initial reported morphology

The morphology from the positive bottle will often determine which antibiotic(s) are utilized by a provider.

Note- if any PCR testing is performed at the time of bottle positivity, it is important that the Gram stain results are reviewed for correlation with the PCR results

Scope time



Lower reproductive tract specimens

BACTERIAL VAGINOSIS

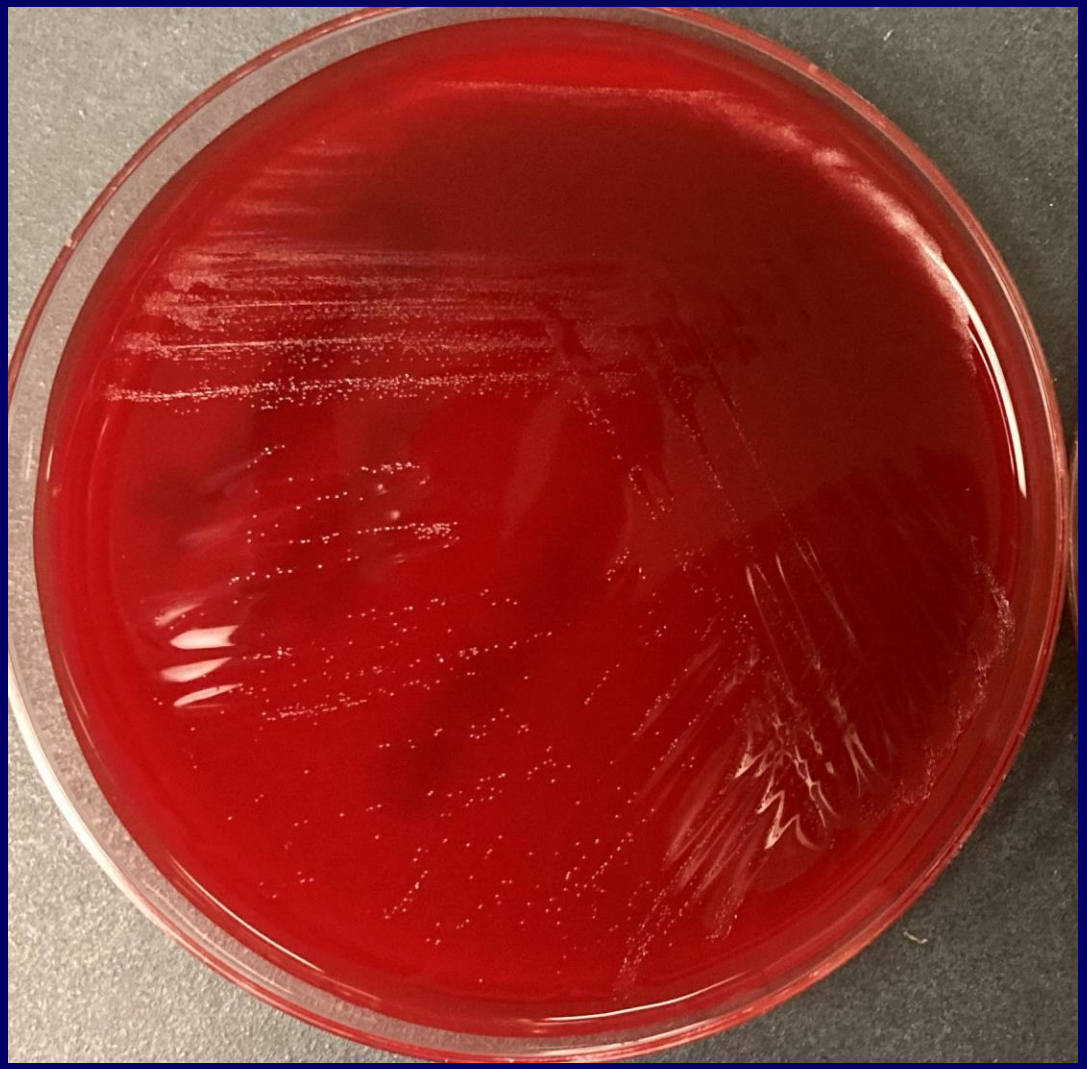
- Dysbiosis of *Lactobacillus* spp. versus…

  Gardnerella spp.
  Atopobium spp.
  Mobiluncus spp.
  Mycoplasma hominis
  Ureaplasma spp.
  Megasphaera spp.

- Significance of *Gardnerella* spp.

  Normal anorectal flora, both genders
  Normal vaginal flora in women of reproductive age
  Teeny tiny colonies

BE CAREFUL



ALTERNATIVES

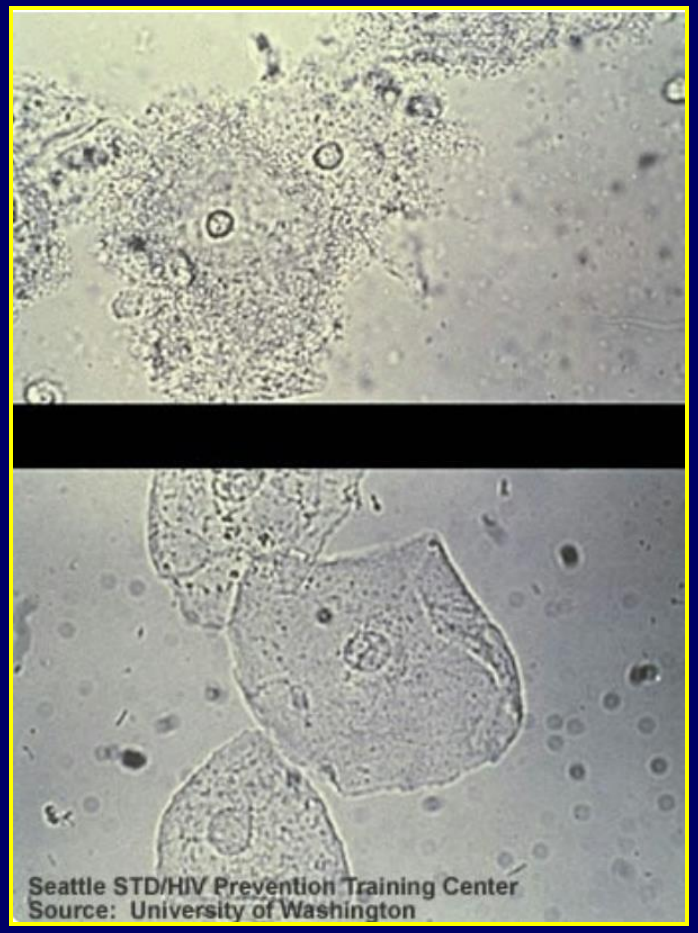
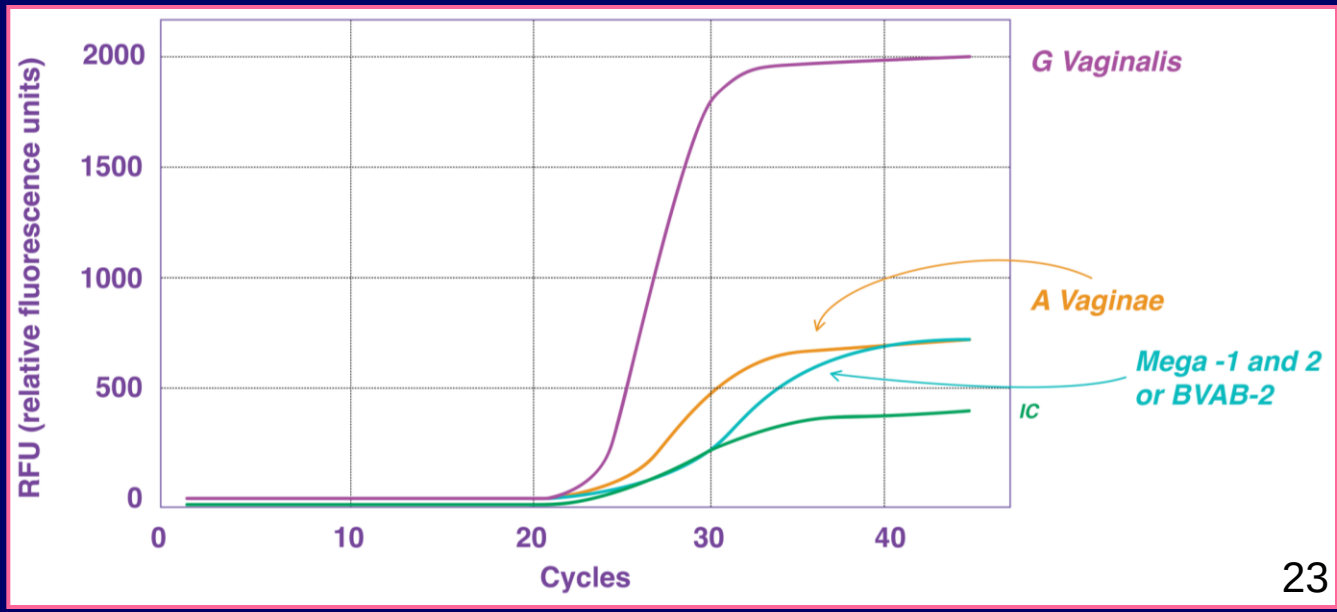


Table 3.2.1–A1 Standardized scoring method for evaluation of Gram stains for BV

Quantitation of bacterial morphotype[a]	Points scored per morphotype[b]				
	None	1+	2+	3+	4+
Long Gram-positive bacilli (consistent with *Lactobacillus*)	4	3	2	1	0
Small Gram-variable bacilli (consistent with *Gardnerella*)	0	1	2	3	4
Curved Gram-negative or Gram-variable bacilli (consistent with *Mobiluncus*)	0	1	1	2	2

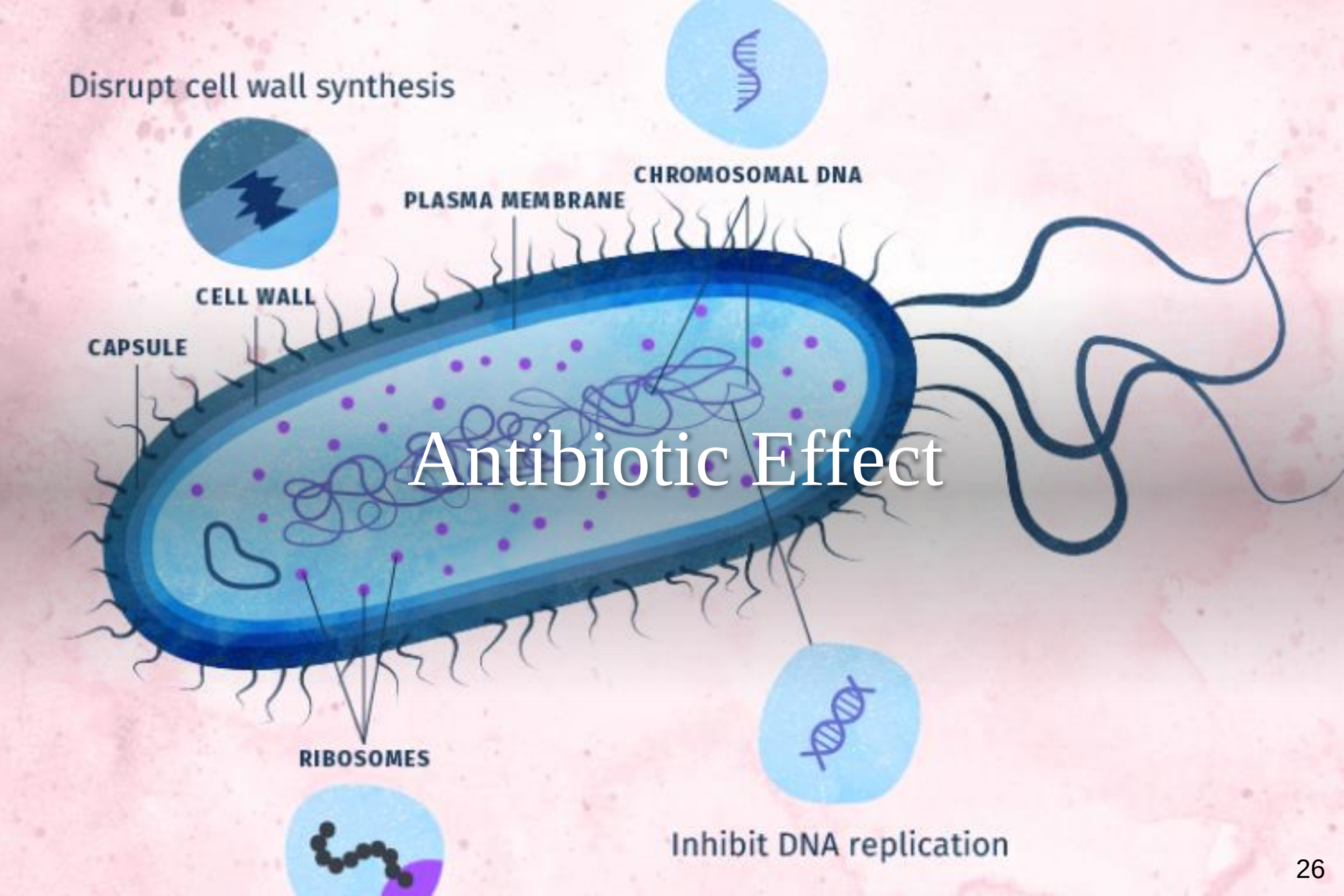
[a]Modified from Nugent et al. (7). Circle in each row the number that corresponds to the quantitation visualized in the smear. Add circled numbers to arrive at the total score. Interpret as follows: 0 to 3, normal; 4 to 6, intermediate; and 7 to 10, abnormal/BV.
[b]1+ = <1 organism/high-power field (HPF); 2+ = 1 to 5 organisms/HPF; 3+ = 6 to 30 organisms/HPF; 4+ = >30 organisms/HPF.

CMPH 5th ed. (vol. 1)



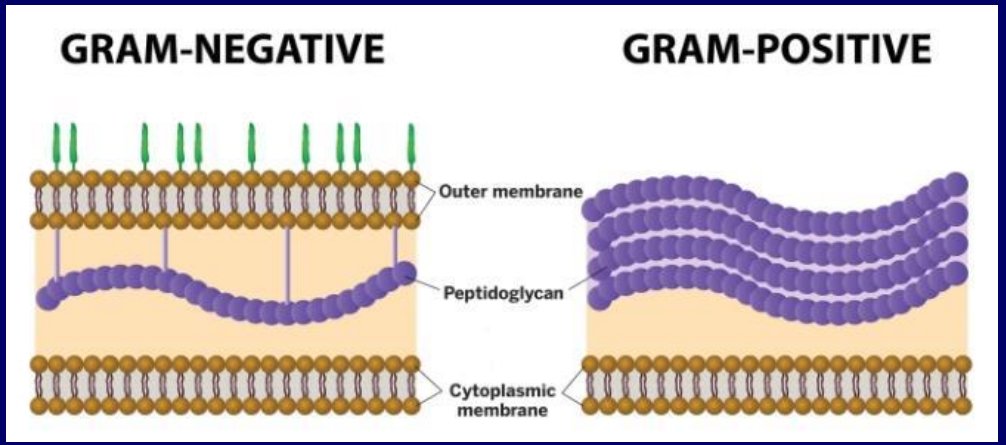
Scope time

POTPOURRI



Antibiotic Effect

Antibiotics typically work by either compromising the cell wall of an organism or disrupting the cell's ability to divide



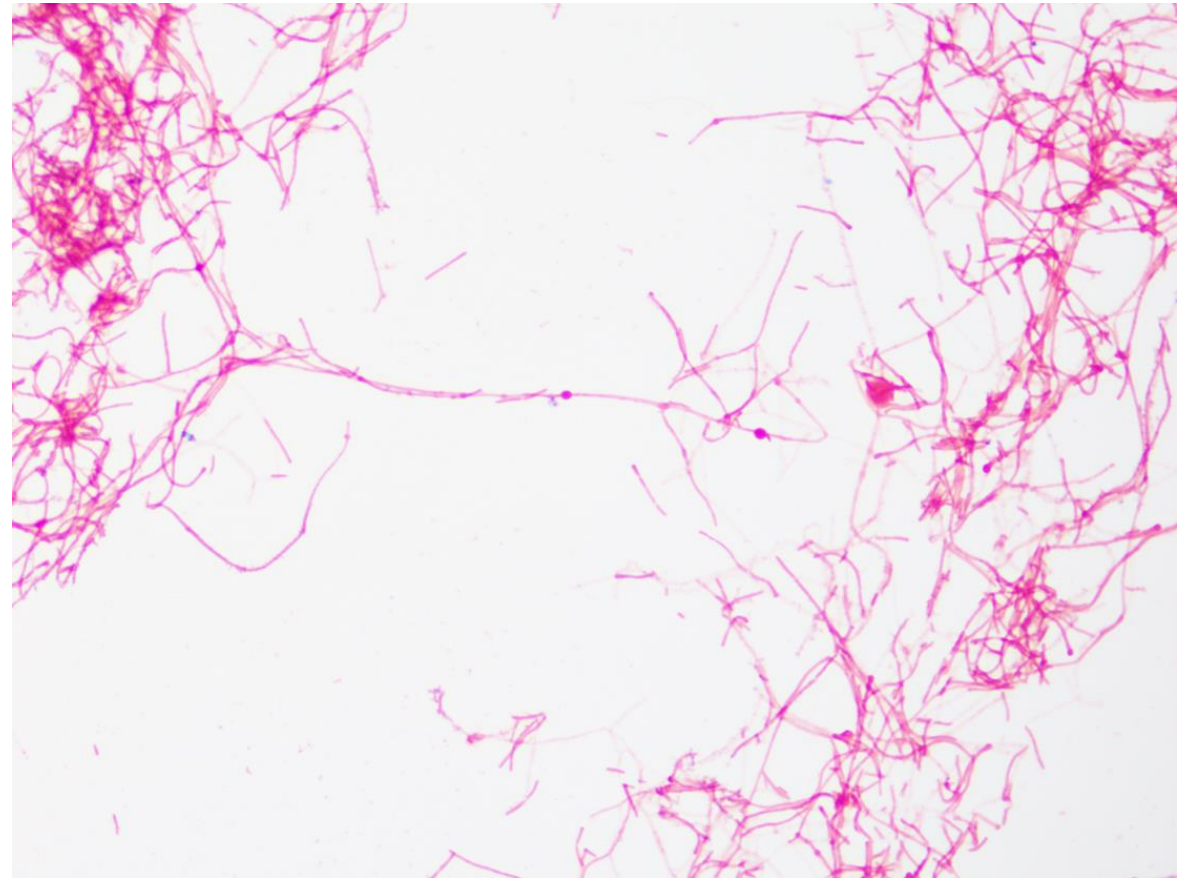
- Depending on the organism and the antibiotic it is exposed to unexpected morphologies are likely to be encountered.

- Gram-positive organisms tend to enlarge and appear Gram-variable to Gram-negative when the production of peptidoglycan is disrupted

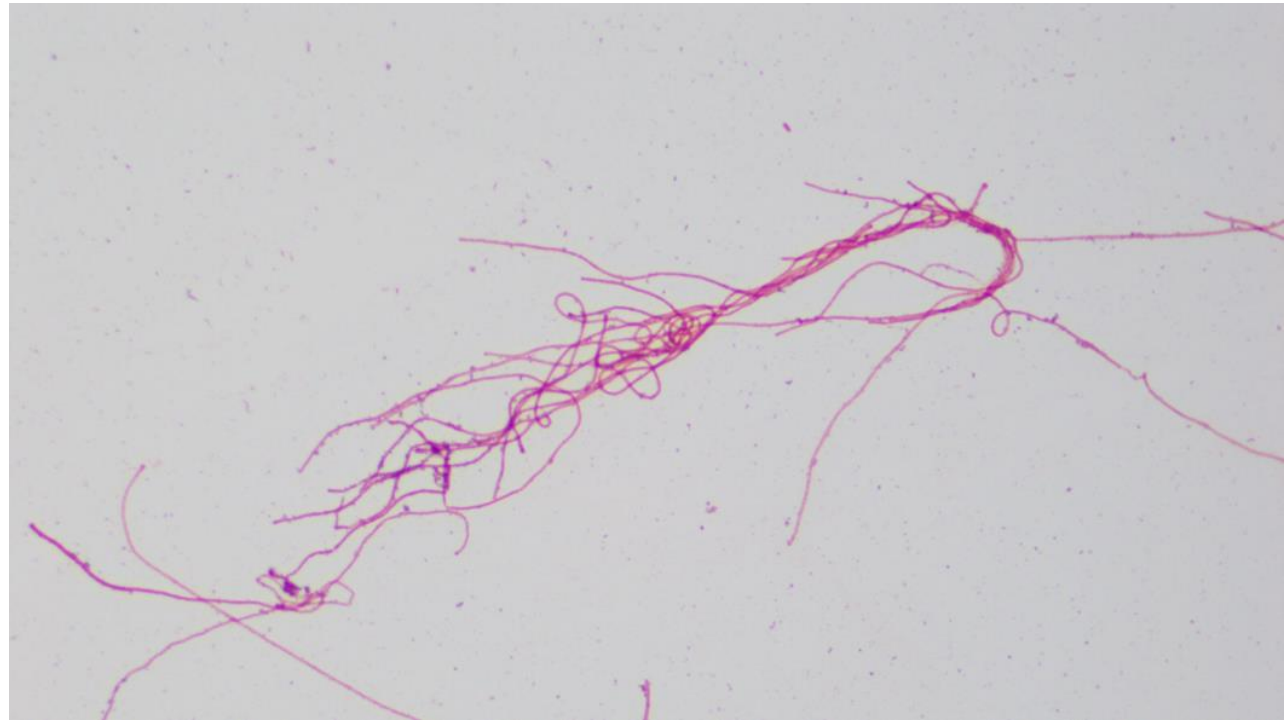
- Gram-negative organisms typically elongate exhibiting filamentous shapes with random thickening

Pseudomonas aeruginosa treated with Cefotaxime

Pseudomonas aeruginosa treated with Meropenem



Pseudomonas aeruginosa treated with a Carbapenem



Scope time

CSF Gram stains

- Typically cytospun Gram stains
- Always keep the big picture in mind and correlate any chemistry results with Gram stain results

	Glucose	WBC	Protein
Normal	40-70 mg/dL (~60% of serum level)	0-30/mm^3	20-150 mg/dL
Acute Bacterial Meningitis	Low	Increased (PMN)	Marked Increase
Early bacterial meningitis	Normal/low	Increased (PMN)	Normal/increased
Listeria	Normal (~20% of cases) Low/normal	Increased (PMN) Increased (lymphs)	Normal/increased Normal/increased
Tuberculosis meningitis	Low	Increased (lymphs)	Normal/ 1 increased
Viral meningitis/encephalitis	Normal	Increased (lymphs)	Normal/increased
Guillian – Barre Syndrome	Normal	Normal	Increased

Scope time