

Culture and identification of infectious agents

Key Terms

After culture

- Biochemical (physiological) tests
- Genetic tests
 - Sequencing,
 - Polymerase chain reaction (PCR)
 - DNA-DNA homology
 - Restriction enzymes (digests)
- Chemical
 - fatty acid/protein profiling
- Immunological

Direct detection (i.e. without culture)

- PCR
- Antigen detection
- Staining (e.g. Gram stain)
- Serology (antibody detection)

- Isolation (culture)
 - Agar plate
plate/colonies
 - Liquid media
- Identification & taxonomy
 - Family
 - Genus
 - Species
 - Type
 - Strain

Taxonomy

- ▶ Defines common traits among strains for a bacterial species
- ▶ Usually genetic
- ▶ Allows development of diagnostic kits

Classification

- ✓ **Strain: one single isolate or line**
- ✓ **Type: sub-set of species**
- ✓ **Species: related strains**
- ✓ **Genus: related species**
- ✓ **Family: related genera**

Steps in isolation and identification

- **Step 1: Streaking culture plates**
 - colonies on incubation (e.g 24 hr)
 - size, texture, color, hemolysis
 - oxygen requirement

Sheep blood agar plate culture

Bacillus cereus.

Bacillus anthracis



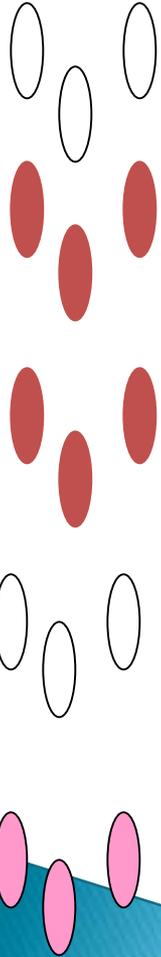
CDC/Dr. James Feeley

Isolation and identification

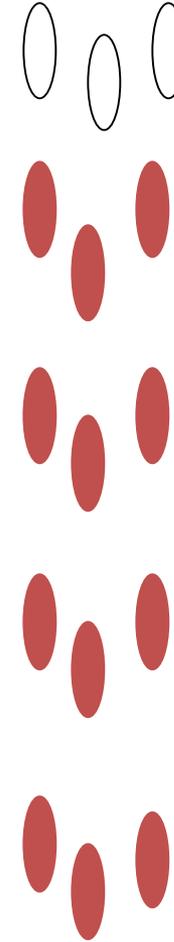
- ▶ **Step 2: Colonies Gram stained**
 - **cells observed microscopically**

Gram Stain

Gram negative



Gram positive



Heat/Dry



Crystal violet stain



Iodine Fix



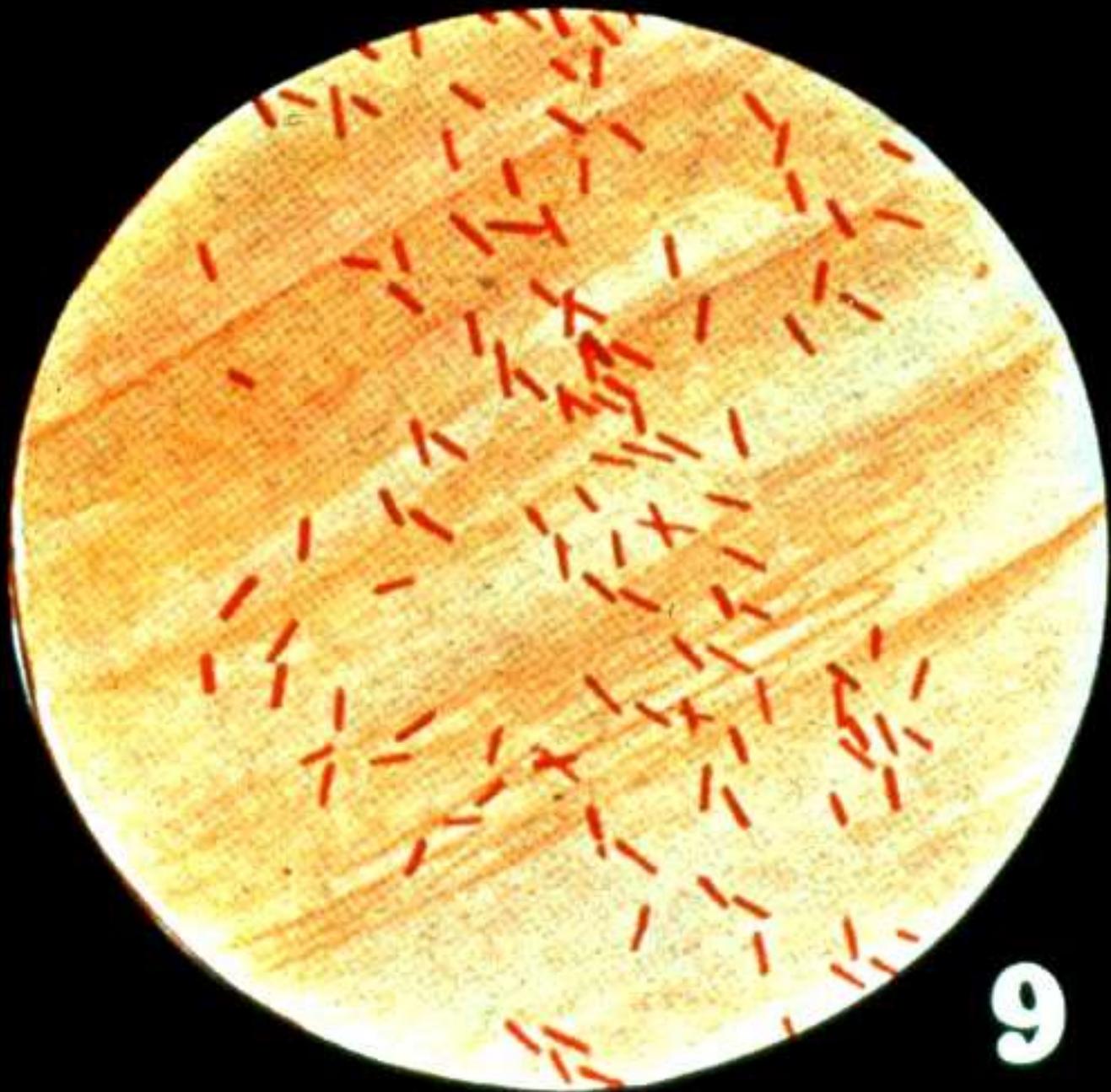
Alcohol de-stain



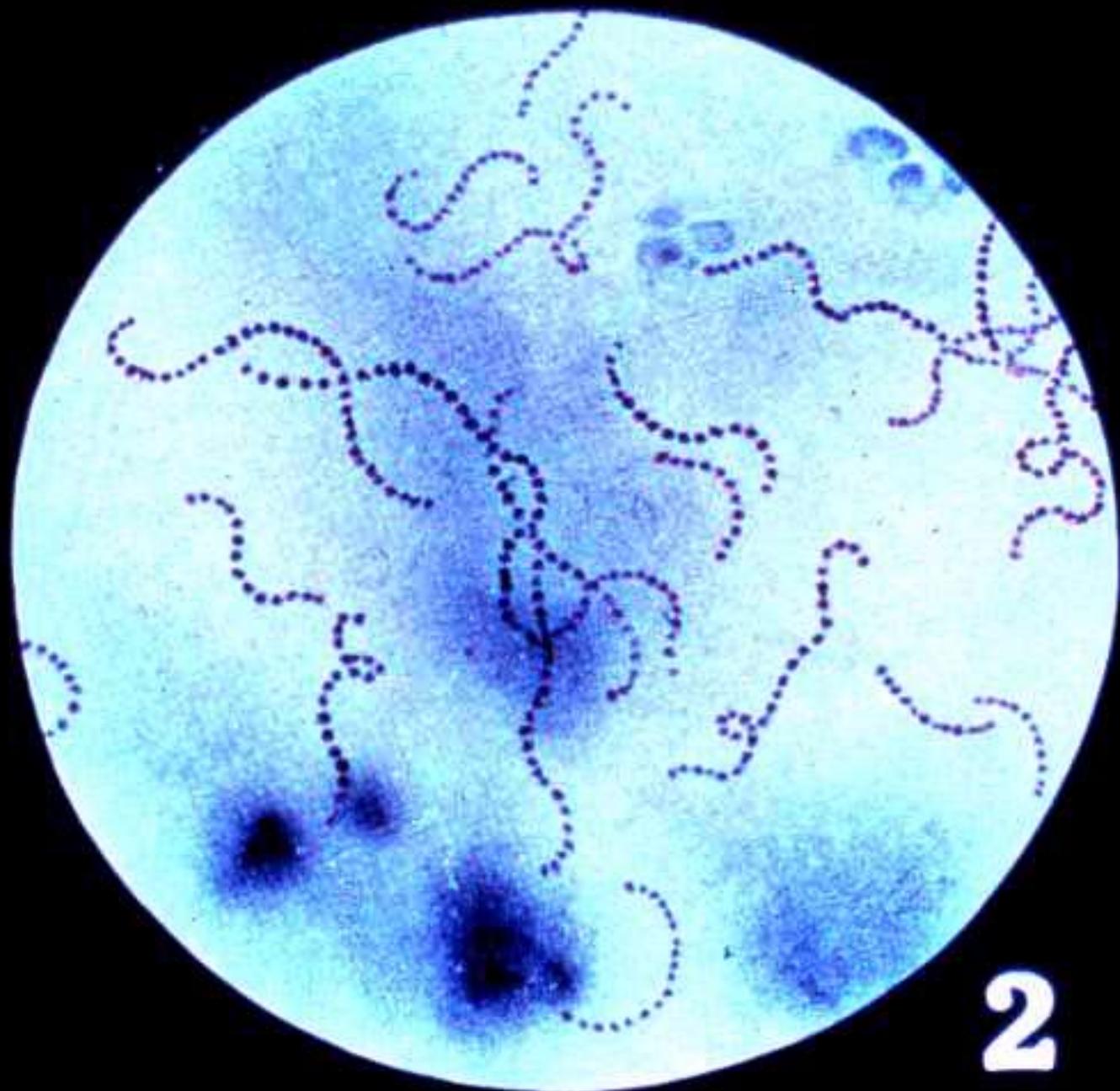
Safranin stain

Gram stain morphology

- ▶ **Shape**
 - cocci (round)
 - bacilli (rods)
 - spiral or curved (e.g. spirochetes)
- ▶ **Single or multiple cells**
 - clusters (e.g. staphylococci)
 - chains (e.g. streptococci)
- ▶ **Gram positive or negative**



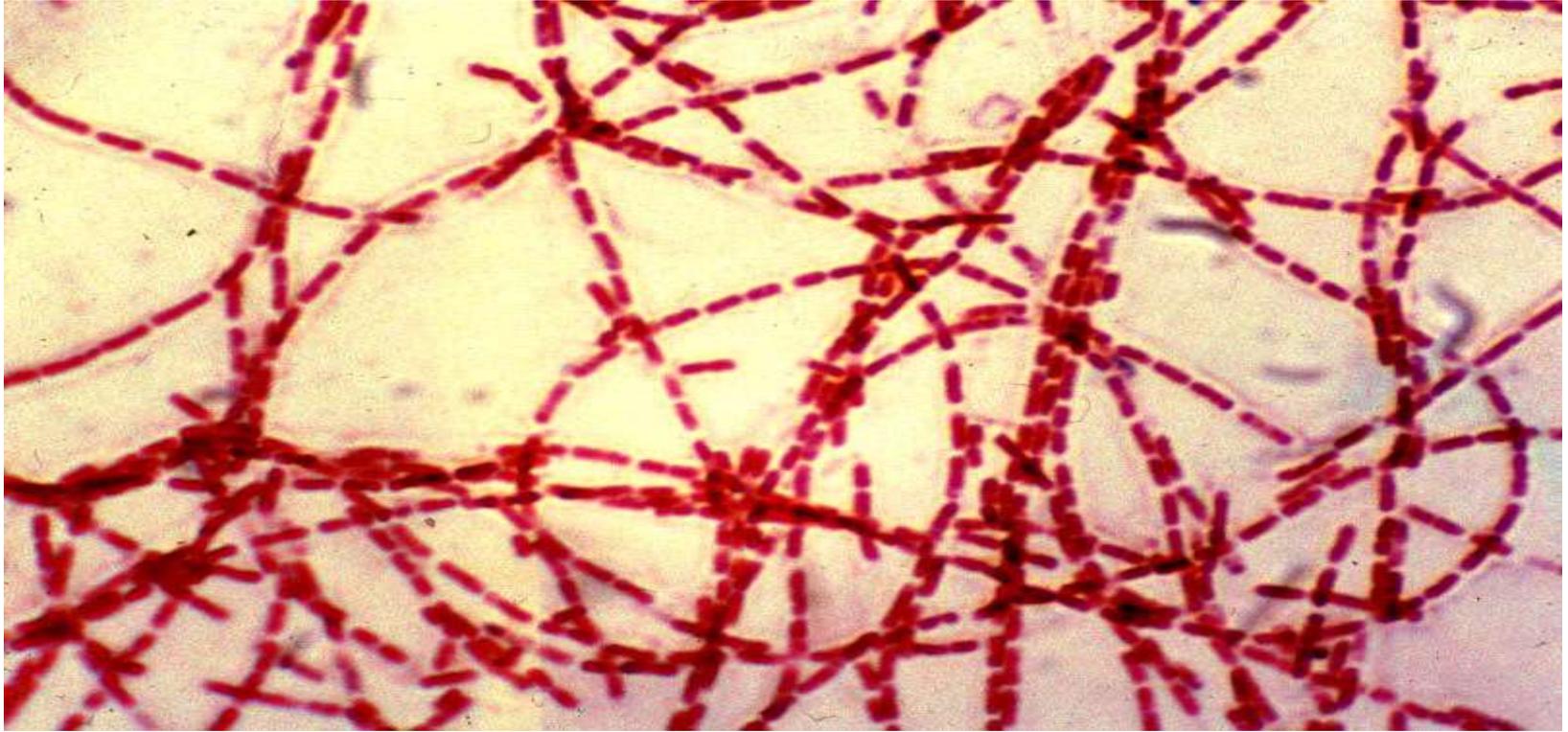
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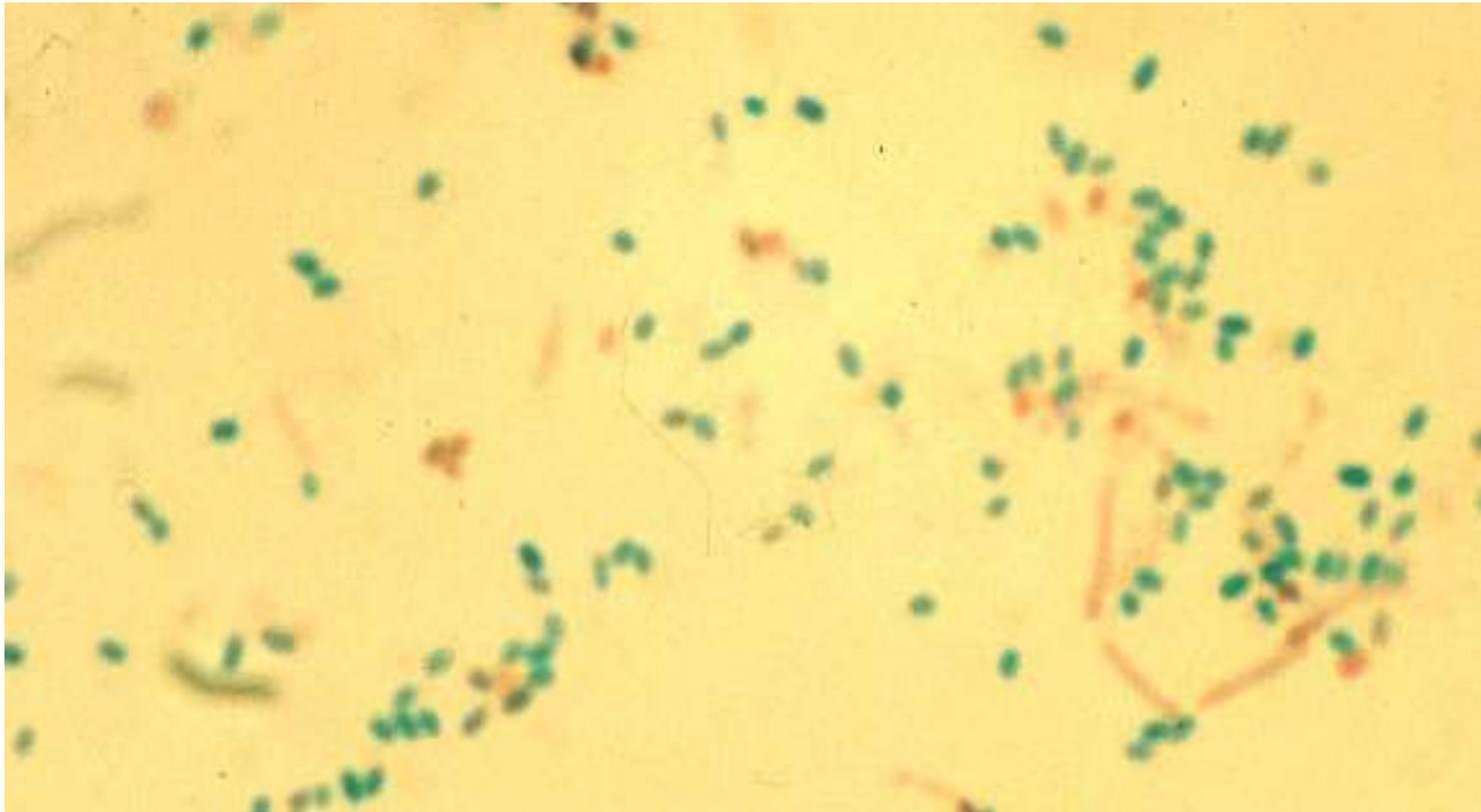
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شريحة نقية

SLIDE



شريحة غير نقية MIXED SLIDE



Step 3:

Isolated bacteria are speciated

- ▶ Generally using physiological tests

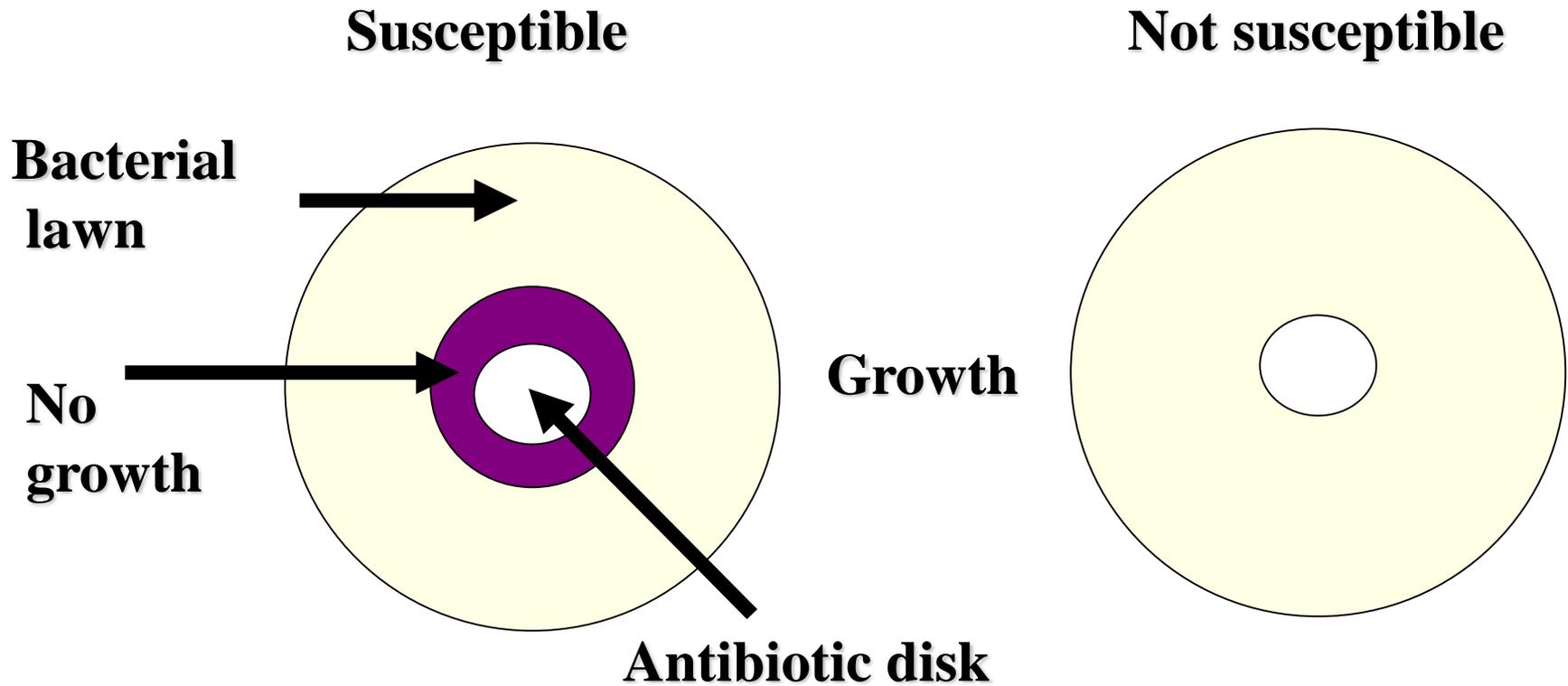
Clinical Microbiology Laboratory Bench



Step 4:

Antibiotic susceptibility testing

Antibiotic susceptibility testing



Molecular differentiation

- **Genomics**
- **Gene characterization**
 - Sequencing
 - PCR
 - Restriction digests
- **Hybridization**
- **% guanine + cytosine**

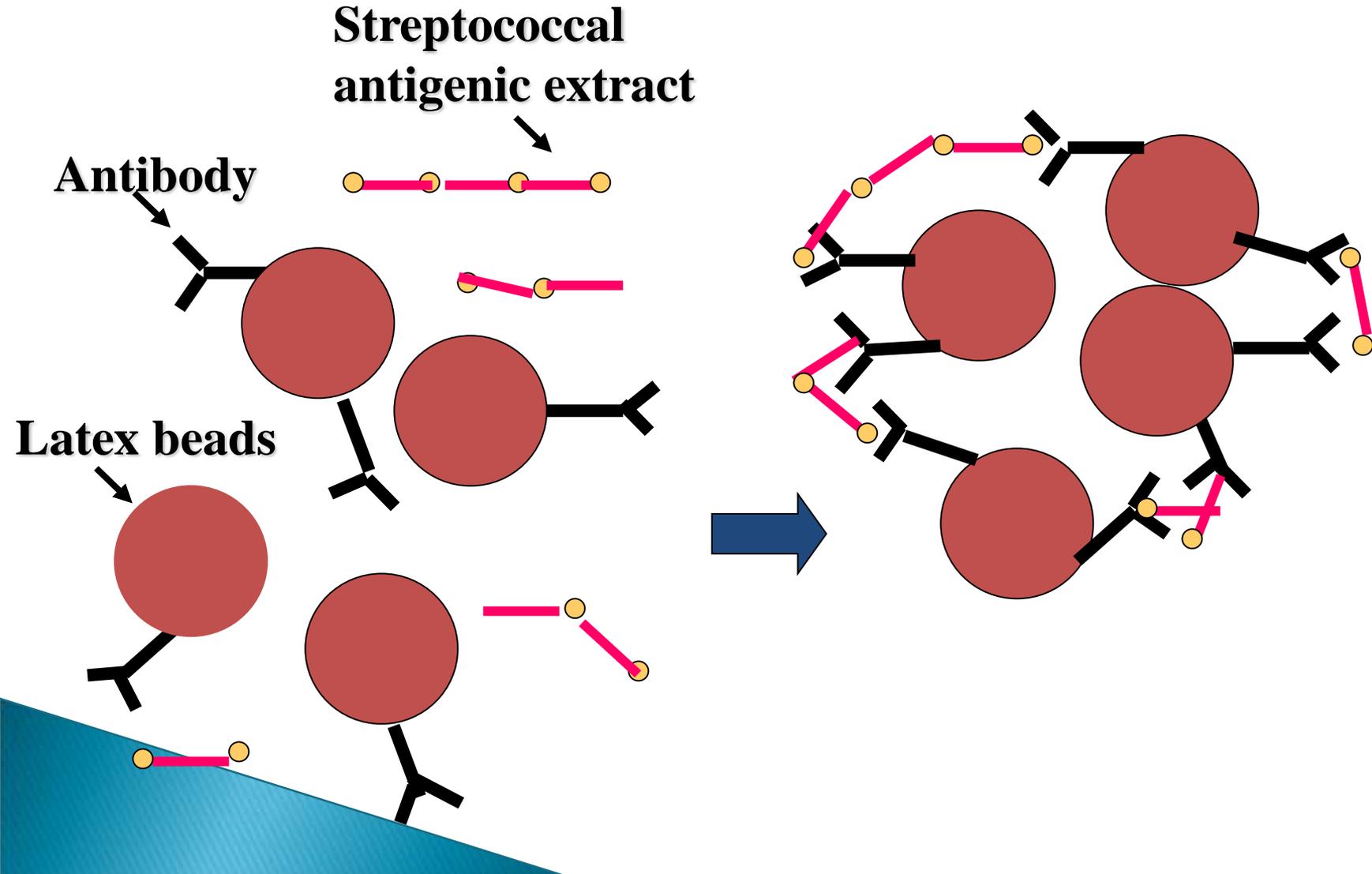
1 6S rRNA Sequencing

- ▶ Differentiates bacterial species
- ▶ Development of clinical tests based on sequence (e.g. PCR)

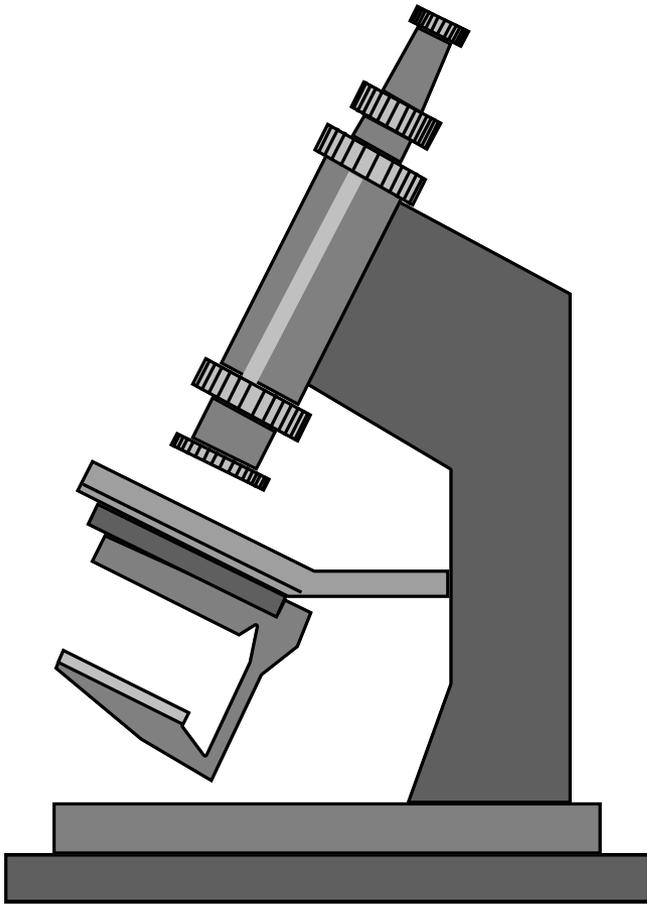
Rapid diagnosis without culture

- **WHEN AND WHY?**
 - **grow poorly**
 - **can not be cultured**

Streptococcal Agglutination Test



Microscopy

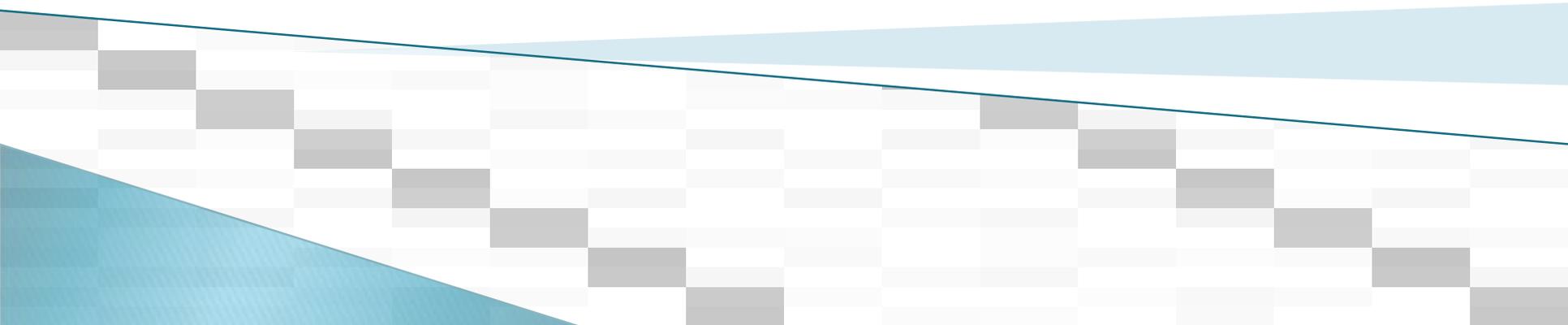


- **spinal fluids
(meningitis)**
- **sputum
(tuberculosis)**
- **sensitivity poor**

Serologic identification

- antibody response to the infecting agent
- several weeks after an infection has occurred

Classification of Bacteria



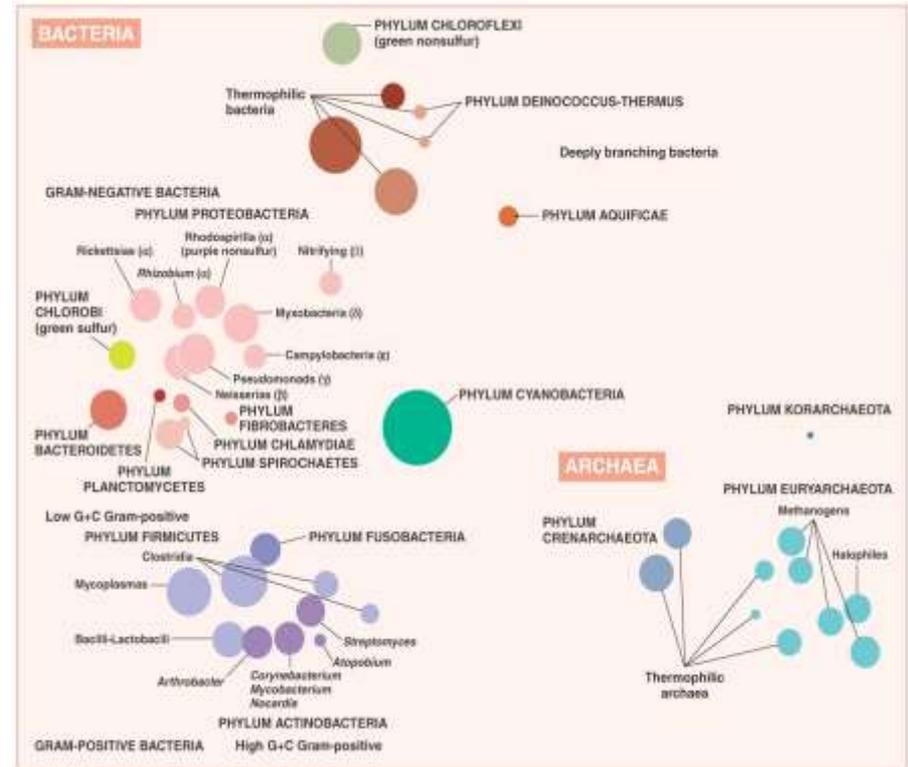
Modern Prokaryotic Classification

Eubacteria

Archeabacteria

Cyanobacteria

Thermophiles



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Diversity of Bacteria



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LM 0.1 mm

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SEM 2.0 μm

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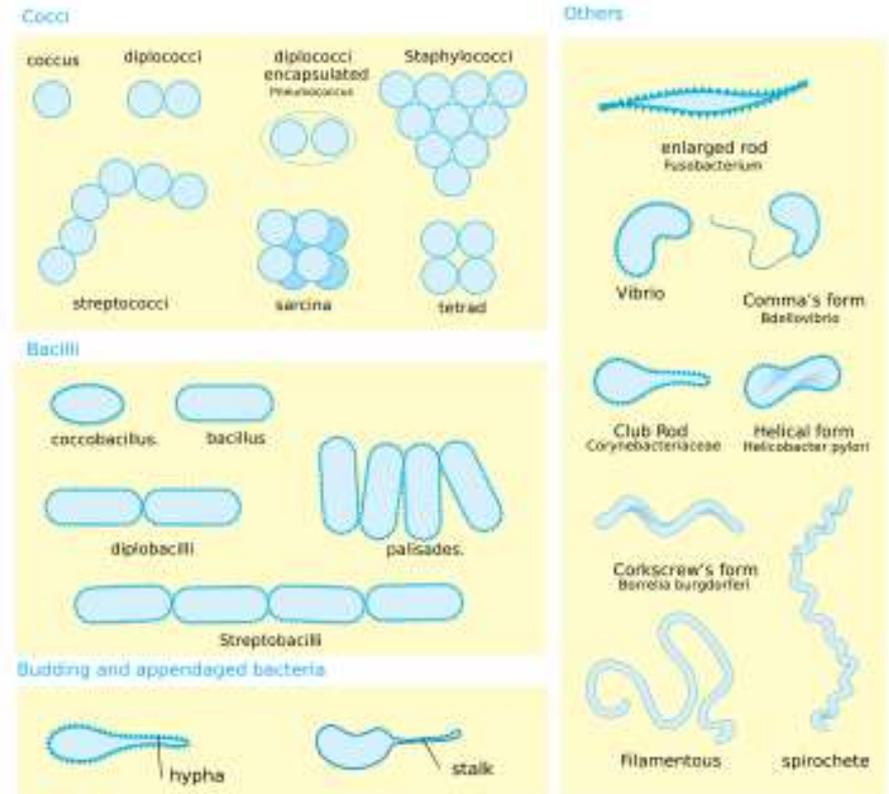
Classification of Bacteria

- ▶ *Classification* – ordering
- ▶ *Nomenclature* – naming
- ▶ Often immortalizes the person who discovered it or its origin
 - *Escherichia coli* → Theodor Escherich
 - coli → from colon
- ▶ *Distinguishing* – identification

Kingdom	Division...	Family	Genus	Species
Procaryotae	Gracilicutes (Gram -)	<i>Pseudomonadaceae</i> (Strictly aerobic, motile straight, or curved rods)	<i>Pseudomonas</i> sp.	<i>P. aeruginosa</i> <i>P. fluorescens</i>
		<i>Enterobacteriaceae</i> (Facultatively anaerobic, straight rods)	<i>Serratia</i> sp.	<i>S. marcescens</i> <i>S. entomophila</i>
		<i>Deinococcaceae</i> (Aerobic cocci, nonmotile)	<i>Melissococcus</i> sp.	<i>M. pluton</i>
	Firmicutes (Gram +)	<i>Bacillaceae</i> (Endospore-forming rods)	<i>Bacillus</i> sp. (Aerobes, facultative anaerobes)	<i>B. alvei</i> <i>B. larvae</i> <i>B. laterosporus</i> <i>B. lentimorbus</i> <i>B. popilliae</i> <i>B. sphaericus</i> <i>B. thuringiensis</i>
				<i>Clostridium</i> sp. (Strict anaerobes)

Classification of Bacteria

- ▶ *Kingdom
- ▶ Phylum
- ▶ Class
- ▶ Order
- ▶ Family
- ▶ *Genus (1st name)
- ▶ *Species (2nd name identifier)



Classification of Bacteria

- ▶ Morphology – shape, color, gram specificity
- ▶ Metabolism
- ▶ Molecular techniques – Forensics, DNA finger prints, RNA, protein analysis

