DCLS

DCLS

Division of

Consolidated Laboratory Services

DGS
DEPARTMENT OF GENERAL SERVICES

Virginia Bioscience Foundation in partnership with Varina High School and the Division of Consolidated Laboratory Services

Bacteria Laboratory Presentation

January 23, 2024

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Tentative Schedule

Time	Activity
10:00 a.m.	DCLS Arrival and Set Up
10:50 a.m.	Introductions
11:00 a.m.	Station 1: Overview of the Lab (All Students)
11:20 a.m.	Begin 1st Station Assignment (2,3,4 or 5)
11:40 a.m.	Begin 2nd Station (2,3,4 or 5)
12:00 p.m.	Begin 3rd Station (2,3,4, or 5)
12:25 p.m.	Scheduled HCPS Break
12:30 p.m.	Begin Last Station Assignment (2,3,4, or 5)
12:50 p.m.	Close-out and Clean-up

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Special Thanks!

- Virginia Bioscience Foundation
  - Eric Rhoades
  - Jim Powers
- Go Virginia Grant
- Varina Center for Environmental Studies and Sustainability
  - Jill Rich
  - Kassandra Epps
  - Cherita Sears
- Varina Center for Communications and Media
- Department of General Services
  - Division of Consolidated Laboratory Services
  - Office of Communications
- Collaborative Teaching and Learning Group, LLC

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Glossary of Terms

January 23, 2024

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Definitions

- Ampule: a sealed glass container containing a liquid or powder
- Aseptic Technique: Procedures used to prevent contamination
- Binary Fission: a process where an organism duplicates its genetic material and then divides into two parts which each new organism “part” receiving one copy of the genetic material
- Biological Metabolism: a series of reactions that occur within living organisms to sustain life
- Differentiation: the act of showing or finding difference between things that are compared (distinguish one microbe from another)
- Don: To put on
- Doff: To take off or remove

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Definitions

- Incubate: to maintain an item for a defined period of time under known or well-defined conditions (ie. temperature)
- Infectious: The ability to cause disease
- Impregnated: Soaked or saturated with a substance
- Lawn of Bacteria: a continuous, uniform layer of bacterial growth on the surface of agar media
- Emulsify: to mix so thoroughly that it becomes an emulsion, or a mixture of two substances that can't be completely blended together
- Homogenous: of uniform or the same structure or composition throughout

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Definitions

- McFarland standards: a series of standards of varying turbidities that allow scientist to compare unknowns to visually to approximate the number of bacteria in a liquid suspension
- Media: liquid or gel that contains nutrients to help organisms grow
- Microbe: a very small living organism that cannot be seen without a microscope
- Morphology: the form and structure of living organisms such as bacteria
- Phenotypic: an observable trait

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Learning Objectives

- Practice effective safety protocols during all stations
- Discuss principles of bacteriology and virology
- Examine laboratory techniques for the identification and differentiation of microbes

Procedural Steps

- Meet your facilitators
- Divide into groups
- Rotate to each station
- Follow station facilitator instructions
- Complete the hands-on activity
- Ask questions
- Record observations and reflections
- Provide feedback to facilitators, teachers, or other guests

Safety

- **Universal precautions:** handle all materials as potentially infectious
- Know the location of eye wash/drench hose
- Wear fully enclosed shoes (heel and toe)
- **Personal Protective Equipment (PPE):** Don (which means to put on) required PPE including, lab coat, safety glasses, and gloves
- Understand hazards associated with chemicals and biological organisms described at each station
- **No food, drink or gum chewing** at stations
- **Wash hands** thoroughly with soap and water

Overview of Stations

- Station 1: Overview
- Station 2: Culture and Sensitivity
- Station 3: Gram Stain
- Station 4: Analytical Profile Index (API) Set-Up
- Station 5: Analytical Profile Index (API) Interpretation

Materials

- Station instructions
- Videos
- Writing utensil (permanent ink not pencil)
- Personal protective equipment (PPE)
  - Lab coat
  - Safety glasses
  - Nitrile gloves

Procedural Steps

- Station 2: Culture and Sensitivity
  - Bacterial cultures
    - Growth media
  - Aseptic technique
  - Colony morphology
    - Size, texture, transparency, pigmentation, elevation, form
  - Antimicrobial resistance

Procedural Steps

- Station 3: Gram Stain
  - Cell walls
    - Chemical and physical properties
  - Gram stain for differentiation
  - Microscopic examination
  - Antibiotics

Procedural Steps

- Station 4: Analytical Profile Index (API) Set-Up
  - Aseptic technique
  - Pipetting
- Station 5: Analytical Profile Index (API) Interpretation
  - Phenotypic microbial identification
  - Biochemical metabolism
    - Color changes
    - Metabolic byproducts (i.e. gases, enzymatic activity)

Resources

- Antimicrobial resistance
  - https://www.cdc.gov/drugresistance/index.html
- Colony characteristics
  - https://www.cdc.gov/labtraining/docs/job_aids/biochemicals_gram_positive_organism_id/Colonial_Characteristics_Branded_508.pdf
- Gram stain
  - https://www.cdc.gov/labtraining/docs/job_aids/biochemicals_and_gram-negative_organism_id/Gram_Stain_Procedure_Branded_508.pdf

Resources

- Culture media
  - https://www.cdc.gov/labtraining/docs/job_aids/biochemicals_and_gram-negative_organism_id/Culture_Media_Table_Branded_508.pdf
- Microbiological identification
  - https://www.cdc.gov/microbenet/about.html
- API
  - https://phil.cdc.gov/Details.aspx?pid=19231

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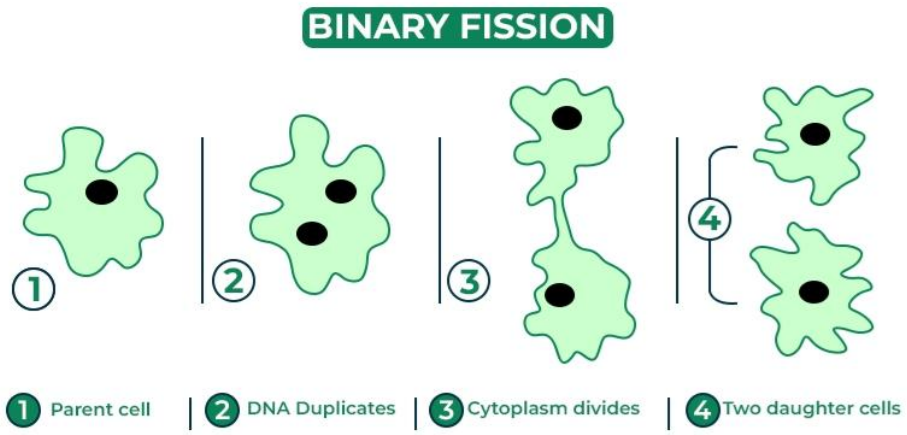
Learning Objectives

- Discuss phenotypic characteristics of bacteria
- Prepare cultures using baker's yeast, not bacteria
- Observe previously prepared bacterial plates to compare phenotypic characteristics and record observations
- Recognize resistance patterns for sensitive versus resistant bacteria

Bacterial Cultures

- Simple cell division by binary fission
- Bacteria multiply once every 20-30 minutes
- Logarithmic growth – in 1 hour a single bacterium can produce 8 or more bacteria
- Aseptic technique
  - Procedures used to prevent contamination
- Growth media (agar)
  - Contents: carbohydrates, minerals, vitamins, proteins

Binary Fission



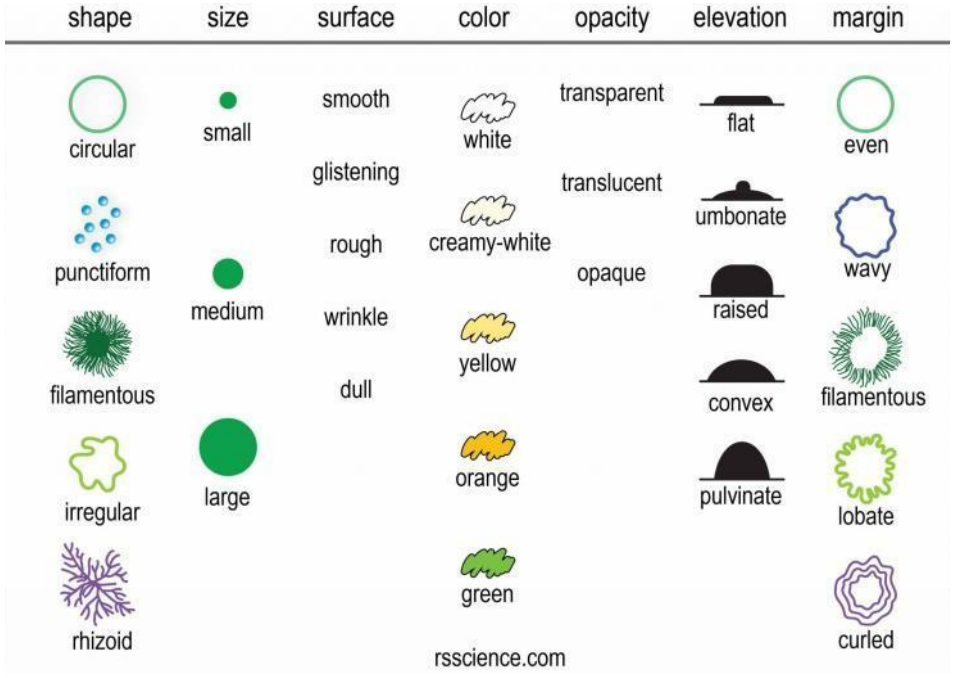
Colony Morphology

- Texture – appearance of the surface
  - Smooth, shiny, mucoid, dry, flaky, etc.
- Transparency – see through them
  - Transparent, translucent, opaque
- Color or pigmentation – intracellular pigments
  - Yellow, pink, purple, red, etc.

Colony Morphology

- Size of Colony
  - Punctiform, small, medium, large
- Elevations
  - Flat, raised, convex, pulvinate, umbonate
- Forms
  - Circular, rhizoid, irregular, filamentous
- Hemolysis
  - Beta, alpha, gamma

Colony Morphology



Safety

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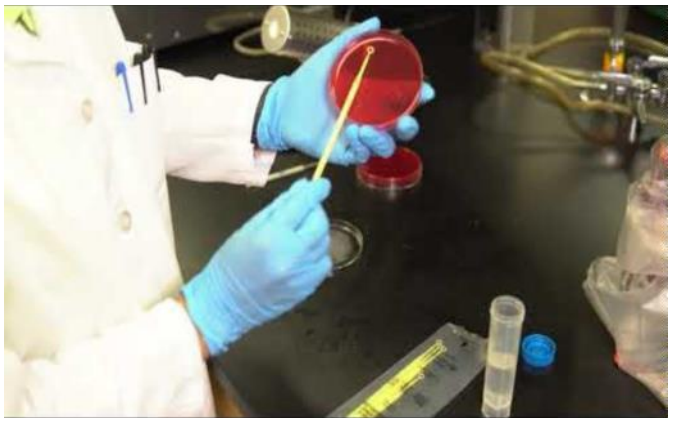
- Know the location of eye wash/drench hose
- Wear fully enclosed shoes (heel and toe)
- **Personal Protective Equipment (PPE):** Don (which means to put on) required PPE including fully enclosed shoes, lab coat, safety glasses, and gloves
- Understand hazards associated with chemicals and biological organisms described at each station
- **No food, drink or gum chewing** at stations
- **Wash hands** thoroughly with soap and water

Inoculation Demonstration



Materials and Reagents

- Gloves
- Inoculation loops
- McFarland standards
- Blood agar plates
- MacConkey plates
- Mueller Hinton plates
- Baker's yeast

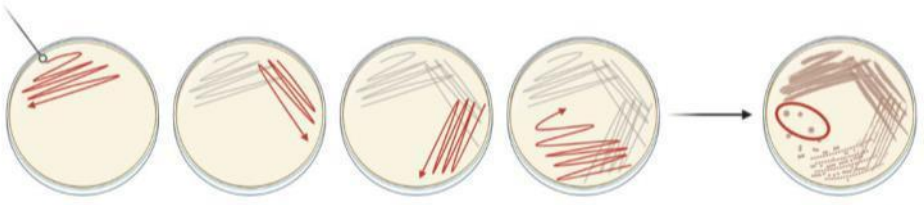
Procedural Steps

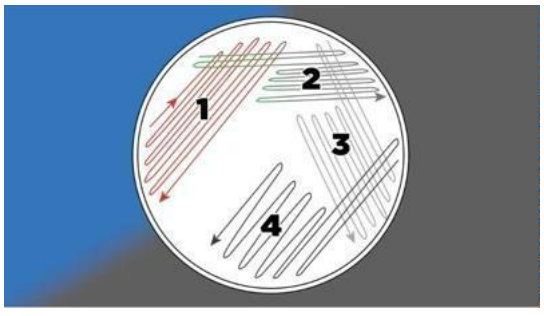
- Don appropriate PPE
- Obtain yeast sample
- Using aseptic technique, retrieve some of the yeast sample with the disposable inoculating loop
- Place the loop flat on the agar surface at the top of the plate and drag in a zig zag pattern for approximately ¼ of the plate
- Discard the used loop and retrieve a new loop

Procedural Steps

- Place the new loop flat on the agar surface in the quadrant that was previously streaked
- Drag the loop in a zigzag pattern for ¼ of the plate
- Discard loop and retrieve a new one
- Repeat this process until all 4 quadrants have been streaked

Four Quadrant Streak Plate Method





Colony Description: MacConkey Agar

Escherichia coli

- Size:_____
- Texture: _____
- Transparency:_____
- Pigmentation: _____
- Elevation: _____
- Form:_____
- Hemolysis:_____

Pseudomonas aeruginosa

- Size:_____
- Texture: _____
- Transparency:_____
- Pigmentation: _____
- Elevation: _____
- Form:_____
- Hemolysis:_____

Colony Description: Blood Agar

- *Staphylococcus aureus*
- Size:_____
- Texture: _____
- Transparency:_____
- Pigmentation: _____
- Elevation: _____
- Form:_____
- Hemolysis:_____

- *Escherichia coli*
- Size:_____
- Texture: _____
- Transparency:_____
- Pigmentation: _____
- Elevation: _____
- Form:_____
- Hemolysis:_____

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Antimicrobial Resistance
Kirby Bauer Disk Diffusion

- Determines whether an organism is sensitive or resistant to an antibiotic
- Used by physicians to make treatment decisions
- Need a "lawn" of bacterial growth and discs impregnated with antibiotics
- Size of the zone of inhibition determines whether a bacteria is sensitive or resistant to that antibiotic
- *Note: different test methods are used for environmental testing*

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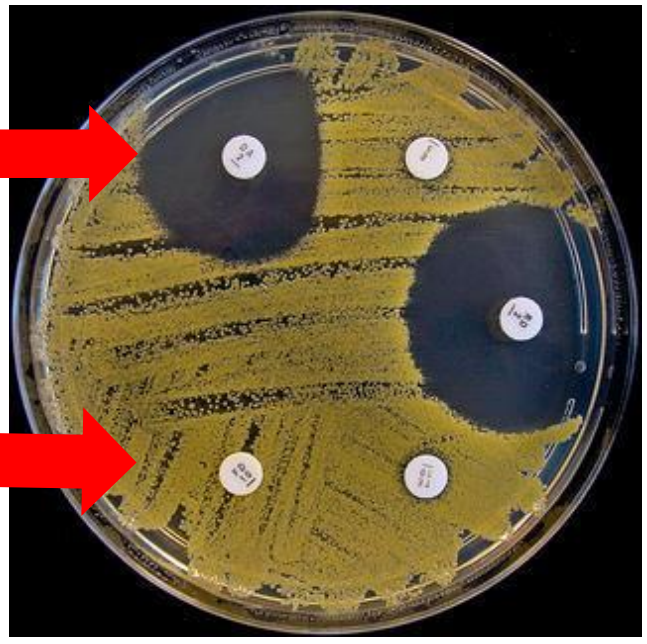
Antimicrobial Resistance Kirby Bauer Disk Diffusion

Sensitive

Resistant



Antimicrobial Resistance -Kirby Bauer Disk Diffusion

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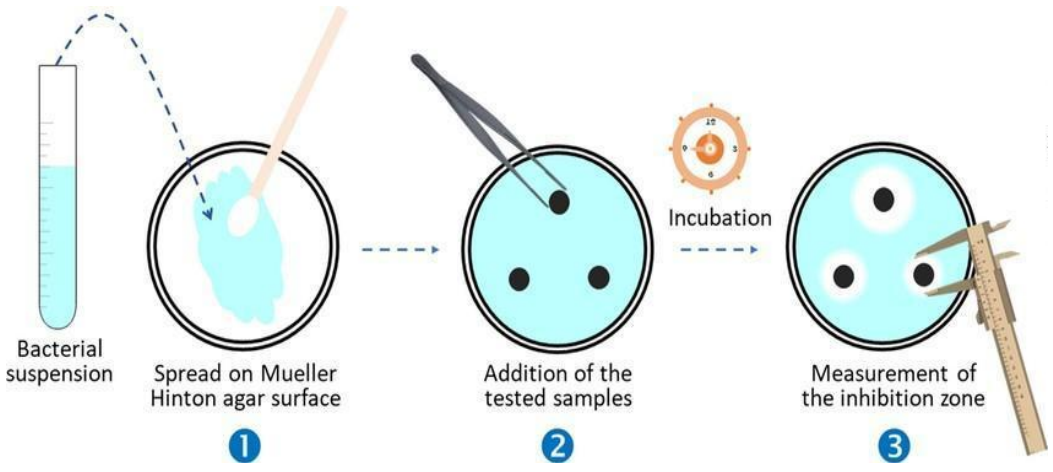
Antimicrobial Resistance
Kirby Bauer Disk Diffusion



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Bacteriology and Virology

Station 3:
Gram Stain

Presenters:

January 23, 2024

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Learning Objective

- Describe the reason for performing gram stains
- Explain 3 outcomes from staining cells and whether they categorize as gram positive, negative, or variable
- Understand the reason why cells stain purple/blue vs red/pink
- Summarize steps for completing a gram stain
- Explain modes of action for antibiotics

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Purpose

- A differential staining procedure to characterize the chemical and physical properties of a cell wall
- Used to differentiate bacteria based on cellular form, size, morphology, and gram reaction
- Guides the laboratory in the selection of appropriate isolation media and tests needed for identification
- Critical test and in some case can serve as the presumptive diagnosis for infectious agents

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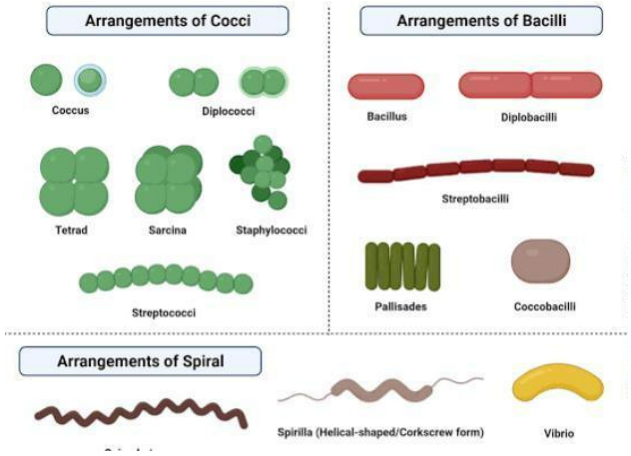
Bacterial Characteristics

- Size
  - 1 to 10 microns – microscopic range
- Morphology



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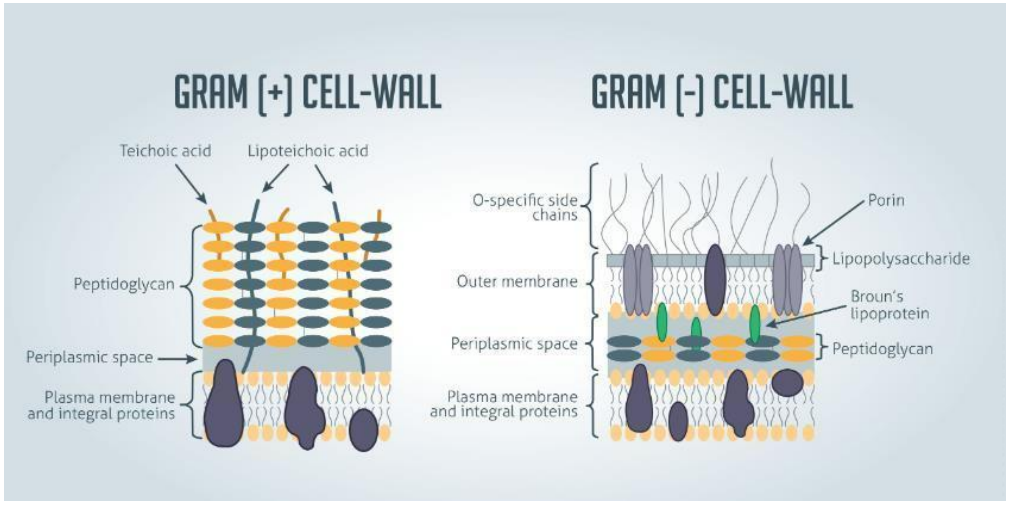
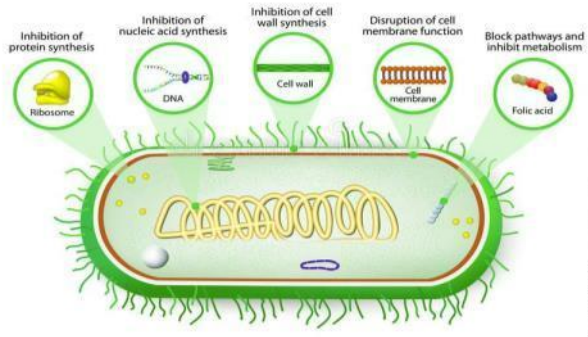
Bacterial Cell Wall Characteristics



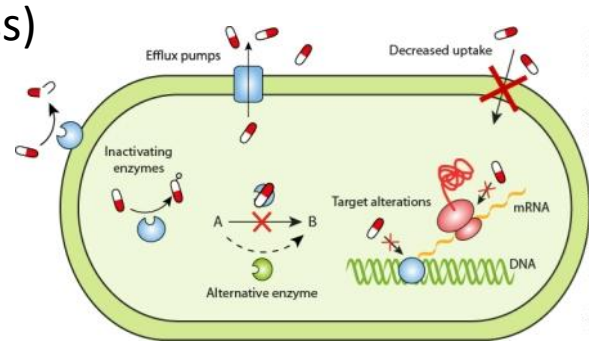
Antibiotics

- Medicines that can kill bacteria *OR* prevent them from multiplying
- 1st Natural Antibiotic – Penicillin (1928)
- Different antibiotics have different modes of action to disrupt bacterial cell growth
  - Interfere with reproduction
  - Block protein production
  - Attack cell wall

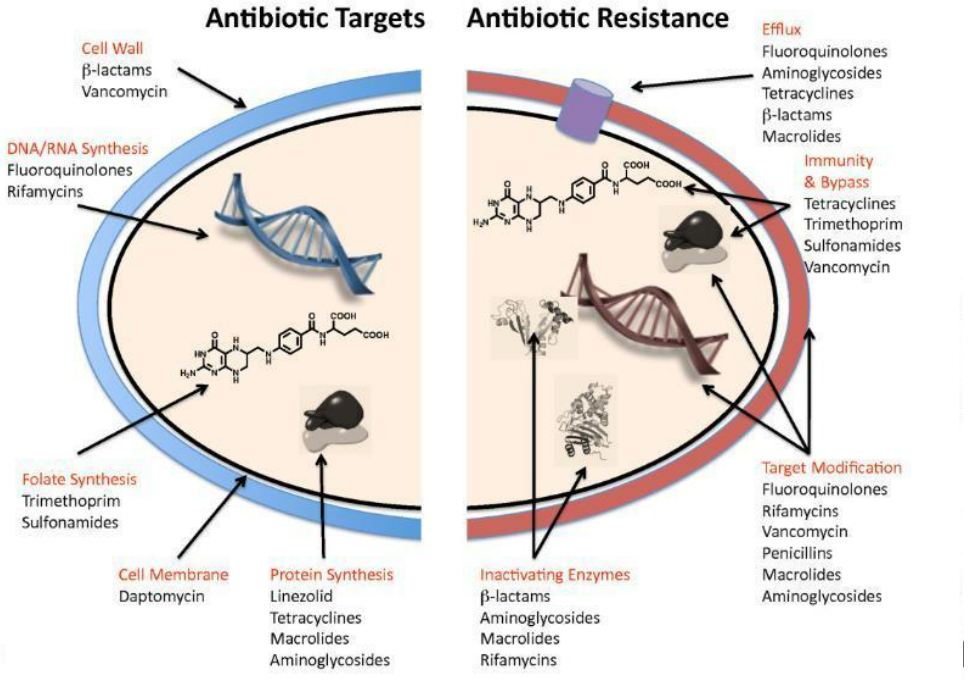


Modes of Resistance

- Many environmental bacteria are multi-drug resistant
- Target modification - Evolution and genetic mutation
- Efflux - Pump out the antibiotics
- Bypass and enzyme-catalyzed destruction - genetic, enzymatic, or replacement of the original target (bypass)



Antibiotics



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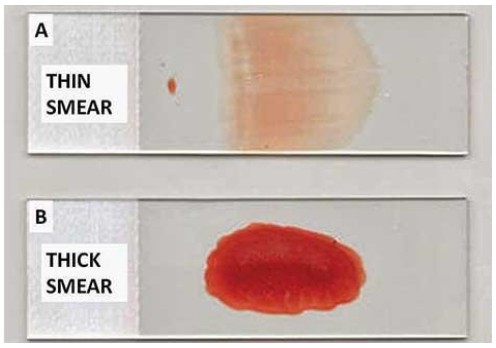
Materials

- Gloves
- Lens paper
- Light microscope
- Prepared gram stain slides
- Immersion oil
- Pasteur pipettes
- Alcohol swabs

Procedural Steps

1. Prepare a thin smear of the material for study on a microscope slide

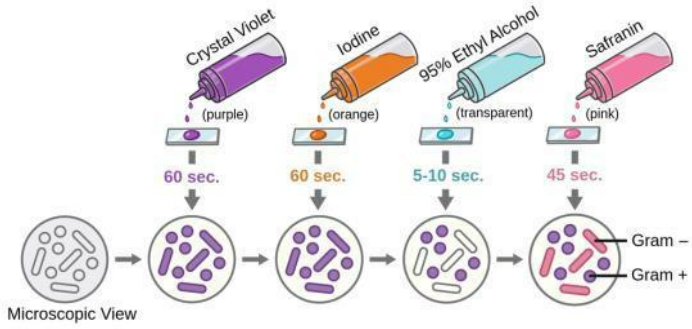
- Place 1-2 drops of broth (organism suspended in media) on a slide using a sterile pipette
- Use a slide warmer to heat fix the smear



Procedural Steps

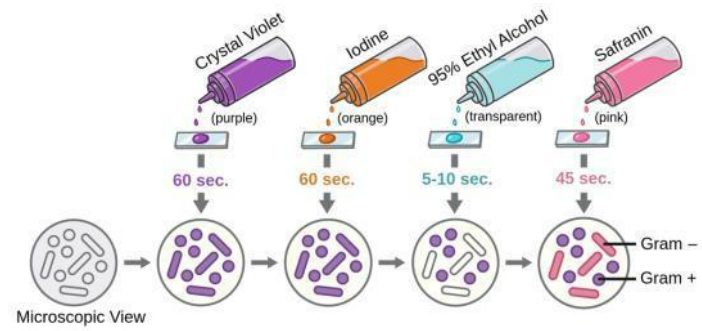
2. Stain the smear

- Place the heat fixed smear on a staining rack over the sink and overlay with Gram Crystal Violet for 1 minute
- Rinse with water and overlay with reconstituted Gram Iodine mordant for 1 minute
- Flood with Gram Decolorizer until the solvent flows colorless from the slide (10-30 sec)

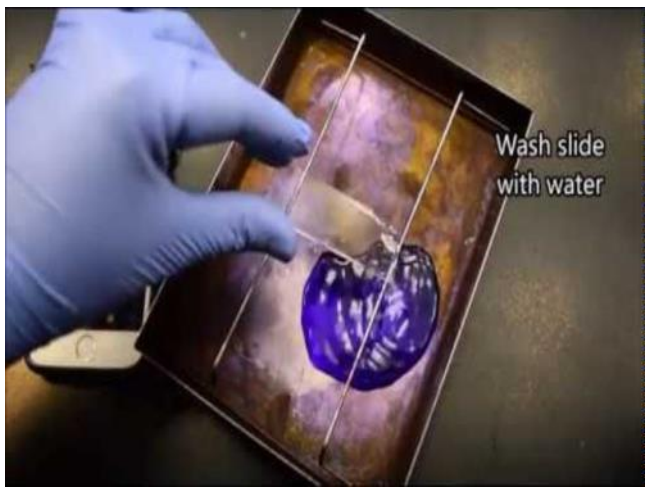


Procedural Steps

- Rinse with water and overlay with Gram Safranin for 30 seconds
- Rinse with water and allow to dry
- Examine the slide microscopically with the 100X oil immersion objective



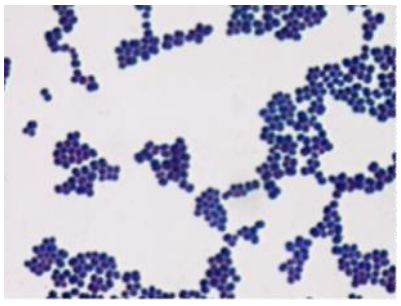
Procedural Steps - Video



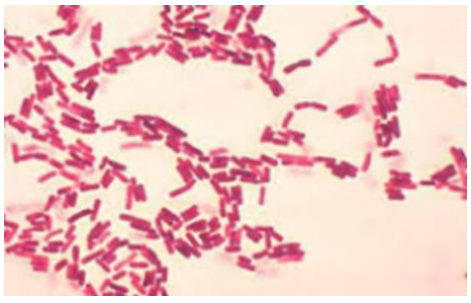
Procedural Steps

3. Interpretation

Gram positive organisms stain deep blue or purple



Gram negative organisms stain red or pink.



Gram variable organisms stain a combination of purple and pink

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Learning Objectives

- Define the purpose of an Analytical Profile Index (API) strip
- Recall different types of organisms that can be identified using API
- Describe biochemicals
- Summarize the steps for preparing an API strip

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Purpose

Rapid and accurate species identification of known, clinically-relevant microorganisms

Gram negative organisms:

- *Neisseria* sp.
- *Haemophilus*
- *Enterobacterales*

Gram positive organisms:

- *Streptococcus* sp.
- Yeasts
- *Staphylococcus* sp.

Biochemicals

Conventional use

Modern Use

Biochemical Testing

- One of the most important methods for phenotypic microbial identification
- Microbes utilize (or metabolize) biochemicals in unique ways, allowing scientists to accurately identify them
- Examples include:

Carbohydrates	Methyl red
Citric acid	Hydrogen sulfide
Indole	Urea

API 20E Strip Biochemicals

- **ONPG**: Detects the enzyme β-galactosidase
- **ADH**: Decarboxylation of the amino acid (aa) arginine by arginine dihydrolase
- **LDC**: Decarboxylation of the aa lysine by lysine decarboxylase
- **ODC**: Decarboxylation of the aa ornithine by ornithine decarboxylase
- **CIT**: Use of citrate as a sole carbon source

API 20E Strip Biochemicals

- **H2S**: Hydrogen sulfide production
- **URE**: Urease enzyme test
- **TDA**: (Tryptophan deaminase): Detection of the tryptophan deaminase enzyme IND: Indole test: production of indole from tryptophan by the enzyme tryptophanase
- **VP**: The Voges-Proskauer test for the detection of acetoin (acetylmethylcarbinol) produced by fermentation of glucose by bacteria using the butylene glycol pathway

API 20E Strip Biochemicals

- **GEL**: Production test of the enzyme gelatinase which liquefies gelatin
- **GLU**: Glucose fermentation (hexose sugar)
- **MAN**: Fermentation of mannose (hexose sugar)
- **INO**: Fermentation of inositol (cyclic polyalcohol)
- **SOR**: Fermentation of sorbitol (sugar alcohol)

API 20E Strip Biochemicals

- **RHA**: Fermentation of Rhamnose (methyl pentose sugar)
- **SAC**: Fermentation of sucrose (disaccharide)
- **MEL**: Fermentation of melibiose (disaccharide)
- **AMY**: Fermentation of amygdalin (glycoside)
- **ARA**: Fermentation of arabinose (pentose sugar)

Safety

- Universal precautions: handle all materials as potentially infectious
- Don appropriate PPE at all times – gloves, lab coat, safety glasses
- Utilize aseptic technique
- Inspect packaging before use to ensure its integrity
- Dispose of any materials that come into contact with the specimen or kit components in biohazardous waste
- No food, drink or gum chewing
- Wash hands thoroughly with soap and water after use

Materials

- Gloves
- Pipettes
- Ampule protector
- Ampule rack
- Incubation box (tray and lid)
- Deionized water squeeze bottles
- Clip seal
- Package insert
- Scissors
- McFarland demonstration tubes

Reagents

- API Test Strip
- API NaCl 0.85% medium
- Mineral oil
- Distilled water

Procedural Steps

- Don appropriate PPE
- Prepare the incubation box and distribute 5ml of distilled water into the honey combed wells of the tray to create a humid atmosphere
- Record the identification information on the box
- Place the gallery in the incubation box under sterile conditions

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Procedural Steps

- Open the ampule of NaCl 0.85%
- Using a swab, remove a single well-isolated colony (18-24 hours old) from the plate
- Carefully emulsify to achieve a homogenous bacterial solution that is 0.5 McFarland
- Immediately use the suspension after preparation (refer to next slide)
- Incubate the tray at 36°C ± 2°C for 18-24 hours

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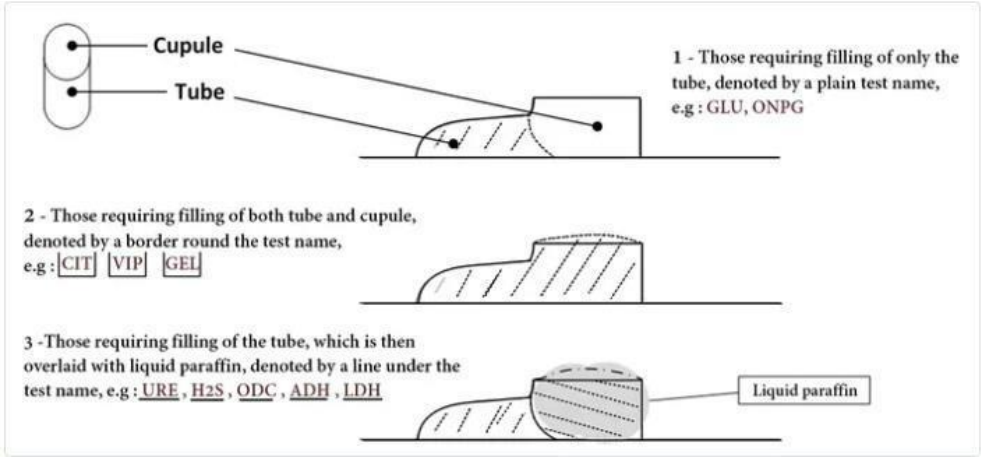
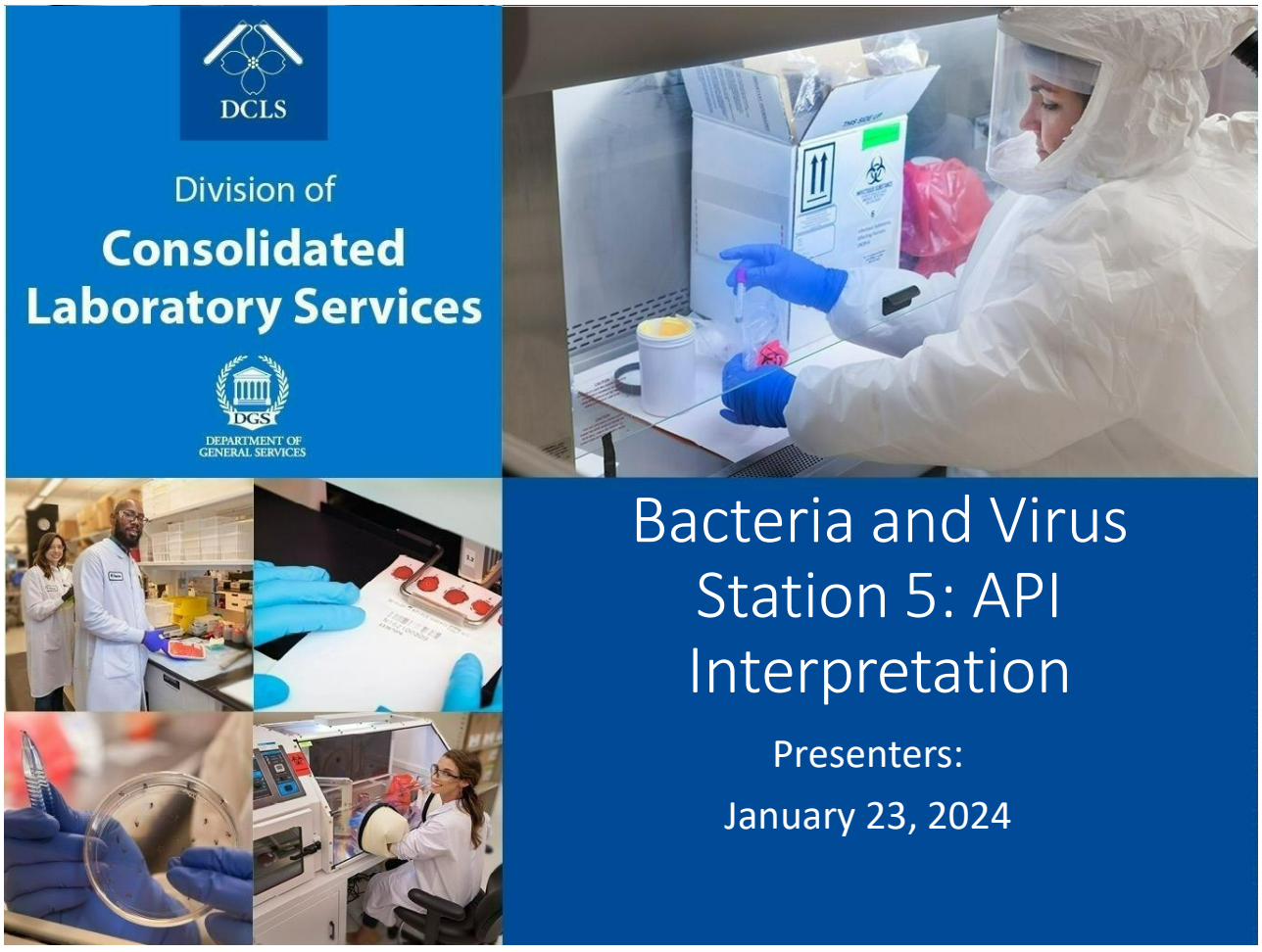
Procedural Steps

Using a sterile pipette, fill the compartments with the bacterial suspension as follows:





Learning Objectives

- Review the API set-up, briefly
- Observe the final steps of the API strip prior to interpretation
- Analyze the reaction of each API well using the reading table
- Interpret the bacterial identification of the strip using the numerical profile

Safety

- Universal precautions: handle all materials as potentially infectious
- Don appropriate PPE at all times – gloves, lab coat, safety glasses
- Utilize aseptic technique
- Inspect packaging before use to ensure its integrity
- Dispose of any material that comes into contact with the specimen or kit components in biohazardous waste
- **No food, drink or gum chewing** at stations
- Wash hands thoroughly after use

Materials

- Gloves
- Pipettes
- Result sheet
- Package insert
- Reading table
- Computer
- APIWEB database (login required)

Reagents

- Prepared API strips
- API reagent kit:
  - TDA
  - James
  - VP1 and VP2

Procedural Steps – Reading the Strip

- Don appropriate PPE – Gloves, safety glasses
- Record all the reactions on the result sheet
  - Except those that require the addition of more reagents (use the reading table)
- TDA test: Add 1 drop of TDA reagent
  - A reddish brown indicates a positive reaction

Procedural steps – Reading the Strip

- VP test: Add 1 drop of VP1 and VP2 reagents. Wait 10 minutes. A pink or red color indicates a positive reaction. Slight pink after 10 minutes should be considered negative
- **Perform the IND test last**: Add 1 drop of JAMES reagent. A pink color developed in the whole cupule indicates a positive reaction

Procedural Steps - Reading Table

READING TABLE

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	2-nitrophenyl-ßD-galactopyranoside	0.223	ß-galactosidase (Ortho NitroPhenyl-ßD-Galactopyranosidase)	colorless	yellow (1)
ADH	L-arginine	1.9	Arginine DiHydrolase	yellow	red / orange (2)
LDC	L-lysine	1.9	Lysine DeCarboxylase	yellow	red / orange (2)
ODC	L-ornithine	1.9	Ornithine DeCarboxylase	yellow	red / orange (2)
CIT	trisodium citrate	0.756	CITrate utilization	pale green / yellow	blue-green / blue (3)
H_2S	sodium thiosulfate	0.075	H_2S production	colorless / greyish	black deposit / thin line
URE	urea	0.76	UREase	yellow	red / orange (2)
TDA	L-tryptophane	0.38	Tryptophane DeAminase	TDA / immediate: yellow	TDA / immediate: reddish brown
IND	L-tryptophane	0.19	INDole production	JAMES / immediate: colorless, pale green / yellow	JAMES / immediate: pink
VP	sodium pyruvate	1.9	acetoin production (Voges Proskauer)	VP 1 + VP 2 / 10 min: colorless	VP 1 + VP 2 / 10 min: pink / red (5)
GEL	Gelatin (bovine origin)	0.6	GELatinase	no diffusion	diffusion of black pigment
GLU	D-glucose	1.9	fermentation / oxidation (GLUcose) (4)	blue / blue-green	yellow / greyish yellow
MAN	D-mannitol	1.9	fermentation / oxidation (MANnitol) (4)	blue / blue-green	yellow
INO	inositol	1.9	fermentation / oxidation (INOsitol) (4)	blue / blue-green	yellow
SOR	D-sorbitol	1.9	fermentation / oxidation (SORbitol) (4)	blue / blue-green	yellow
RHA	L-rhamnose	1.9	fermentation / oxidation (RHAmnose) (4)	blue / blue-green	yellow
SAC	D-sucrose	1.9	fermentation / oxidation (SACcharose) (4)	blue / blue-green	yellow
MEL	D-melibiose	1.9	fermentation / oxidation (MELibiose) (4)	blue / blue-green	yellow
AMY	amygdalin	0.57	fermentation / oxidation (AMYgdalin) (4)	blue / blue-green	yellow
ARA	L-arabinose	1.9	fermentation / oxidation (ARAbinose) (4)	blue / blue-green	yellow
OX	(see oxidase test package insert)		cytochrome-OXidase	(see oxidase test package insert)	

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Procedural Steps - Interpretation

- Identification is obtained with a numerical profile
- Look at the results sheet and visualize that the tests are separated into groups of 3 and a value of 1, 2 or 4 is indicated for each.
- Add together the values corresponding to a positive reaction within each group to obtain a 7-digit profile number for the 20 tests
- The oxidase reaction constitutes the 21st test and has a value of 4 if positive

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Procedural Steps - Interpretation

- If using the database: look up the numerical profile in the list of profiles
- If using the identification software: Enter the 7-digit numerical profile manually into the computer