

Practical 6

Identification of Streptococci & Enterococci

Name:----- Date: \ \2025

***Gram Reaction:** These are the Catalase negative Gram positive cocci in chains , pairs or singles

***Sample Collection:** The type of specimen depends on the suspected infection site:

- **S. pyogenes** - Throat swab (pharyngitis), **Wound swab/Pus** → (necrotizing fasciitis)
- **S. pneumoniae** - Blood/CSF (meningitis, pneumonia), Sputum/BAL (LRTI)
- **S. agalactiae** - Vaginal/rectal swab (neonatal screening)
- **Enterococcus spp.** - Urine/blood culture (UTIs, endocarditis)

***Growth Conditions**

***S. pyogenes & S. agalactiae** - Facultative anaerobe, grows at 37°C, 5-10% CO₂ prefers enriched media (Blood Agar)

***S. pneumoniae & Viridans streptococci** - Facultative anaerobe, grows at 35-37°C with 5% CO₂, prefers Blood Agar and Chocolate Agar.

***Enterococcus spp.** - Facultative anaerobe, grows at 35-37°C, bile salts, survives in 6.5% NaCl

Colonial Morphology

Species	Colony Appearance on Blood Agar tolerates	Hemolysis Type
	Beta hemolytic streptococci	
S. pyogenes	Small, transparent, beta-hemolysis	β (Complete)
S. agalactiae	Medium, grayish-white, narrow β-hemolysis	β (Narrow)
	Alpha hemolytic streptococci	
S. pneumoniae	Small, dome-shaped (young), flattened (older), alpha-hemolysis	α (Partial)
Viridans streptococci	Small, greenish colonies, alpha-hemolysis	α (Partial)
	Enterococci	
Enterococcus spp.	Small, raised, opaque, non-hemolytic or alpha-hemolysis	α/γ (Variable)

Culture Media for Isolation: CLED or Blood agar according to site of infection>

Biochemical Tests for Identification of Enterococcus faecalis:

1. Bile Esculin Hydrolysis Test

Aim:

To determine the ability of the organism to grow in the presence of bile and hydrolyze esculin.

Principle:

Enterococcus faecalis can hydrolyze esculin in the presence of bile. When esculin is hydrolyzed, it produces **esculetin** and **glucose**. Esculetin reacts with ferric citrate in the medium to form a **black complex**.

Requires: **Bile Esculin Agar** plate or slant.

Steps:

1. Inoculate the bile esculin agar slant or plate with the test organism.
2. Incubate at 35–37°C for 24–48 hours.

Results & Interpretation:

- **Positive:** Blackening of more than half the medium → *Enterococcus faecalis* likely.
- **Negative:** No color change or slight darkening only near the growth line → Not *E. faecalis*.

2. Salt Tolerance Test (6.5% NaCl):**Aim:**

To test the organism's ability to grow in high salt concentrations (6.5% NaCl), which helps distinguish *Enterococcus* from other Gram-positive cocci.

Principle:

Enterococcus faecalis can survive and multiply in environments with high salt concentration, unlike many other bacteria. Growth results in **turbidity** of the broth.

Steps:

1. Inoculate the organism into a tube containing Brain Heart Infusion (BHI) broth with 6.5% NaCl.
2. Incubate at 35–37°C for 24–48 hours.
3. Observe for turbidity.

Results & Interpretation:

- **Positive:** Visible turbidity or growth → *Enterococcus faecalis* likely.
- **Negative:** Clear broth (no growth) → Not *E. faecalis*.

4. Litmus milk reduction test:

This test is a quick and affordable method for identifying enterococci. It relies on the ability of enterococcal species to reduce litmus milk, changing its color from mauve to white or pale yellow due to enzymatic activity.

Requires: Litmus milk broth:

Steps:

1. Inoculate 0.5 ml of sterile litmus milk with a heavy inoculum from the test organism (scrape three times from a dense culture).

- Incubate at 35–37°C for up to 4 hours, checking every 30 minutes for a color shift from mauve to white or pale yellow. Compare with a positive control.

Results Interpretation:

- White or pale yellow** = Indicates Enterococcus presence
- No change or pink** = Suggests it's not Enterococcus

Test	Result
Catalase	Negative
Bile Esculin Hydrolysis	Positive
Salt Tolerance (6.5% NaCl)	Positive
Litmus Milk	+ve Acid production & milk reduction
-The isolated organism according to the performed tests is <i>Enterococcus faecalis</i>	

***Just for your knowledge: Antimicrobial Susceptibility Testing (AST)

Performed using Kirby-Bauer Disk Diffusion

Species	First-Line Antibiotics	Resistant Strains Treatment
S. pyogenes	Penicillin, Amoxicillin	Macrolides (Erythromycin) if allergic
S. agalactiae	Penicillin, Ampicillin	Vancomycin (if resistant)
S. pneumoniae	Penicillin (if sensitive), Ceftriaxone	Vancomycin (for resistant strains)
Viridans streptococci	Penicillin, Ceftriaxone	Vancomycin (if resistant)
Enterococcus spp.	Ampicillin, Vancomycin	Linezolid, Daptomycin for VRE

Lab report

A 68-year-old female suffering from type 2 Diabetes Mellitus, hypertension, recent urinary catheterization following hospitalization for hip surgery, came to the physician complaining of: Burning sensation during urination, Increased frequency and urgency, Suprapubic pain, Mild fever (38.1°C) suggestive of Urinary tract infection.

Routine Laboratory Findings of urine sample

- Macroscopic Observation: **Color:** Cloudy, **Odor:** Foul
- Microscopy (Urine Wet Mount): Numerous **WBCs** and few **RBCs**, Direct Gram stain: **Gram-positive cocci in pairs and short chains**

A midstream (clean catch) urine sample was submitted to the lab for culture and sensitivity, perform the identification tests and antimicrobial sensitivity test for the isolated organism in CLED agar:

Colonial morphology: -----

Gram reaction:-----

Requirement for cultivation and Identification tests:

Day 2 (Overnight incubation):

----- The isolated organism according to the performed tests is: -----

Practical 7

Bacillus species identification

***Samples Collection:**

B. cereus: Stool samples (within 24 hours of symptom onset), Vomitus, Suspected food (leftovers or batch samples) Also Wound swab or pus aspirate & Blood.

B. Anthracis: Swab of the vesicle or eschar (before crust formation), Punch biopsy, Blood, Sputum (if productive), Bronchoalveolar lavage (BAL).

***Microscopy:**

Gram reaction: Bacillus species are Gram positive rods often arranged in pairs or chains with rounded or square ends and usually have a single endospore.

B. cereus is usually motile haemolytic and penicillin resistant, while *B. anthracis* is nonmotile, non hemolytic and penicillin sensitive.

***Growth Conditions:**

- They are aerobic or facultatively anaerobic, and most species are motile (a notable exception is *Bacillus anthracis*) by peritrichous flagella. Most species are **oxidase positive**, which may lead to confusion with *Pseudomonas*.
- Blood agar incubated in air/CO₂ at 35°C-37°C for 24 – 48hr
- On Blood agar **colonial morphology**: Medium to large (2–5 mm), flat, dry, irregular, White. Beta-hemolytic (clear zone).

Biochemical (Identification test):

- **Catalase +ve & Oxidase +ve** tests
- **Motility**: All *Bacillus* species are motile except *B. anthracis*.
- **Penicillin susceptibility**: Should be performed on the well Area on a streaked blood agar to check hemolysis and sensitivity to penicillin. All *Bacillus* species, except *B. anthracis*, are generally resistant to penicillin (≤ 28 mm inhibition zone diameter).

Lab report

A 45-year-old male, a chronic smoker, with recent trauma from a motor vehicle accident. Visited the doctor after having sustained a deep laceration on the right leg during the accident, requiring stitches.

The patient Suffered from Pain, swelling, and redness around the wound site, Increased warmth over the wound area, and Purulent discharge (yellowish-green) from the wound, indicating Wound infection.

A wound swab was submitted to the microbiology lab for Identification of the causative agent. You are provided with **Nutrient agar** to perform the identification of the isolate.

This image shows a full page of blank handwriting practice paper. It features multiple sets of horizontal lines, each set consisting of three dashed lines to define the height of capital letters and a single solid line at the bottom for lowercase letters. The sets are repeated down the entire page, providing ample space for practicing letter formation and alignment.