Bacterial Identification and Classification - Laboratory Approaches

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Outline

- Brief review- approaches to bacterial classification

- Introduction to Biochemical Tests

- Categories of Biochemical Tests:

  - Primary Biochemical Tests

  - Secondary Biochemical Tests

  - Automated Identification Systems

Approaches to Bacterial Classification

Traditional/Phenotypic Methods:
- **Morphology (shape)**
- **Staining: Gram stain, Acid-fast stain**
- **Culture requirements: oxygen requirements, temperature, pH**
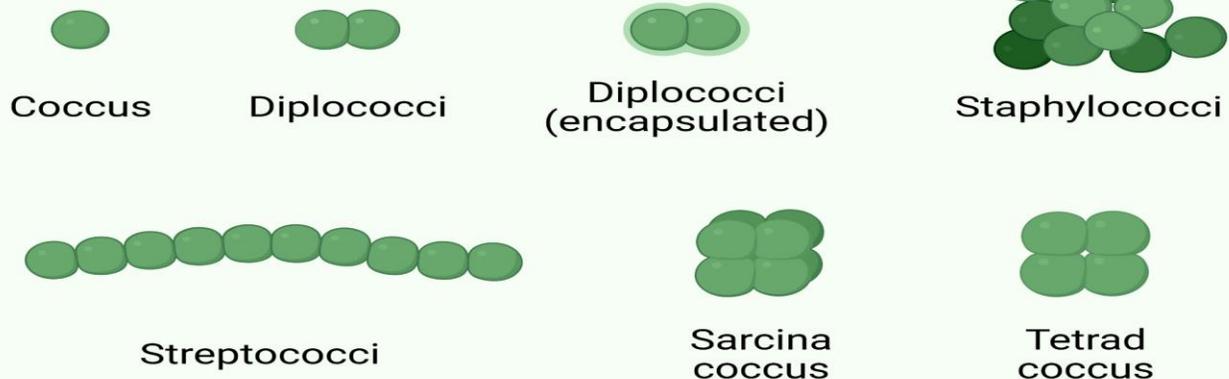- Biochemical characteristics: Sugar fermentation tests

Modern (Genotypic & Molecular) Approaches
- DNA G+C content
- 16S rRNA sequencing
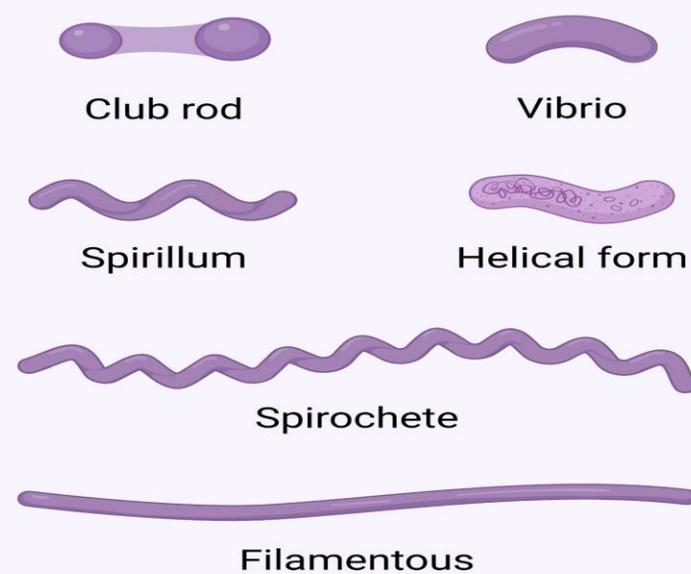- Whole genome sequencing, phylogenetics
- MALDI-TOF
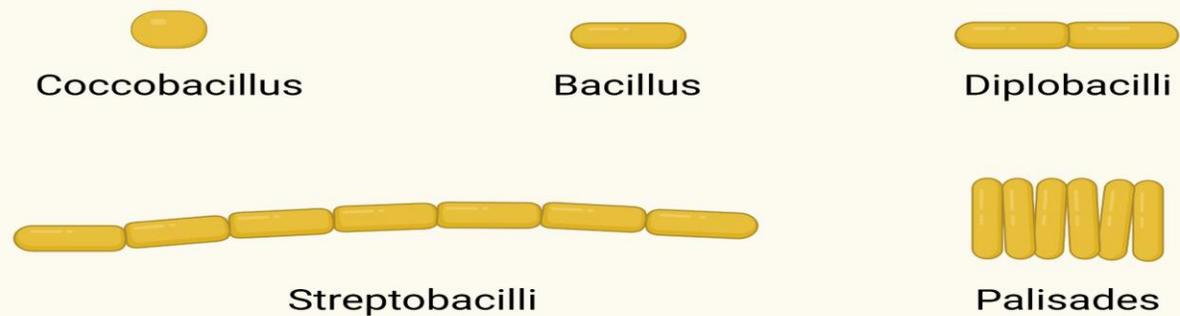
Bacterial Shapes and Arrangements

Traditional Approaches to Bacterial Classification: Gram Stain

- **Differential stain** - divides bacteria into two major groups based on their reaction to the staining procedure

- **Two groups:**

  Gram-positive bacteria → retain primary stain → appear **Purple/Blue**

  Gram-negative bacteria → lose primary stain → appear **Pink/Red**

Gram Positive Bacteria
(stains purple)

Gram Negative Bacteria
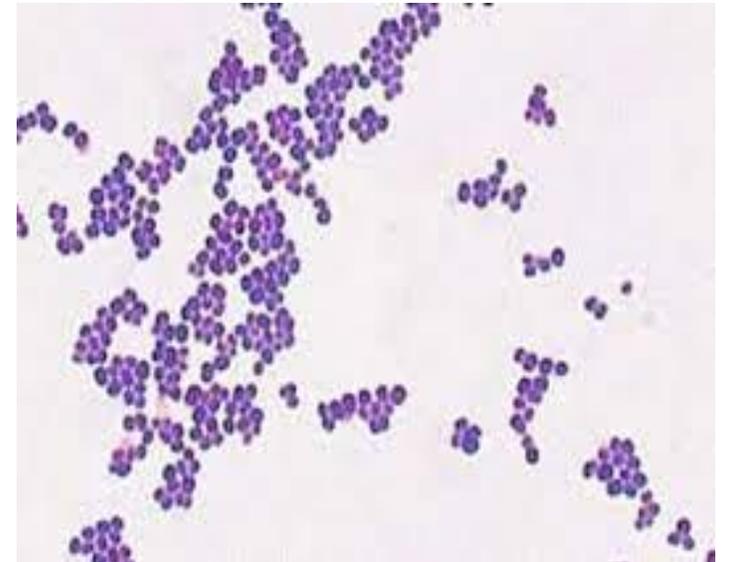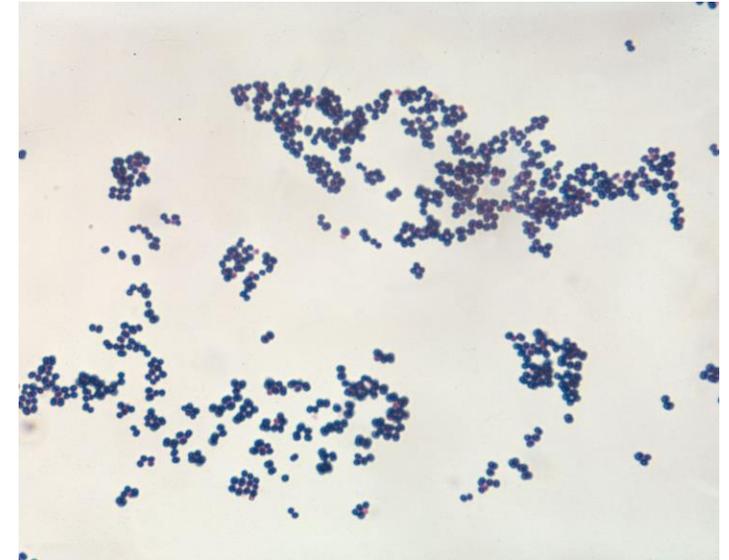(stains red/pink)

Gram Staining
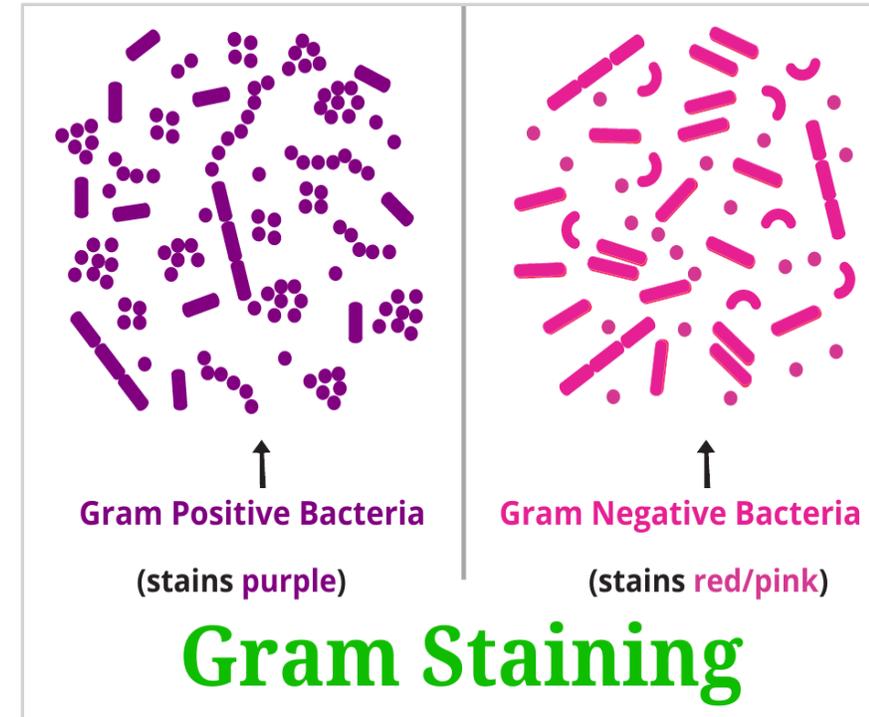
Biochemical Tests - Introduction

But This Is NOT Enough!

❌ **Example:** Both *Staphylococcus aureus* and *Staphylococcus epidermidis* are:

- Gram-positive cocci in clusters
- Look identical under microscope!

➡️ We need **BIOCHEMICAL TESTS** to differentiate them

Biochemical Tests - Introduction

Biochemical Tests are tests that detect **bacterial enzymes** and **metabolic activities** to identify and differentiate bacterial species.

Why Do We Need Them?

- **After Gram Stain, we know:**
  - Gram-positive or Gram-negative
  - Shape and arrangement
- **But we still need to answer:**
  - Which specific genus?
  - Which specific species?
  - Which strain (sometimes)?

Biochemical Tests - Introduction

How Do They Work?

Bacteria differ in their ability to:

✓ Produce specific enzymes

✓ Ferment different sugars

✓ Break down proteins/amino acids

✓ Produce gases (H_2S, CO_2)

✓ Utilize different nutrients

→ Each test detects **ONE specific metabolic activity**

→ Combination of results = Bacterial **"biochemical profile"**

Biochemical Tests Categories

Categories of Biochemical Tests:

- **Primary/Rapid tests** (minutes to hours): Catalase, Coagulase, Oxidase

- **Secondary tests** (24-48 hours): Sugar fermentation, Urease, and H2S production

- **Automated systems** (hours): API strips, VITEK

Biochemical Tests: Primary Biochemical Tests- Catalase test

Purpose: Differentiates catalase-positive from catalase-negative bacteria

Principle: Detects enzyme **catalase** that breaks down $H_2O_2 \rightarrow H_2O + O_2$

Method:

- Add hydrogen peroxide to bacterial colony on slide and observe immediately
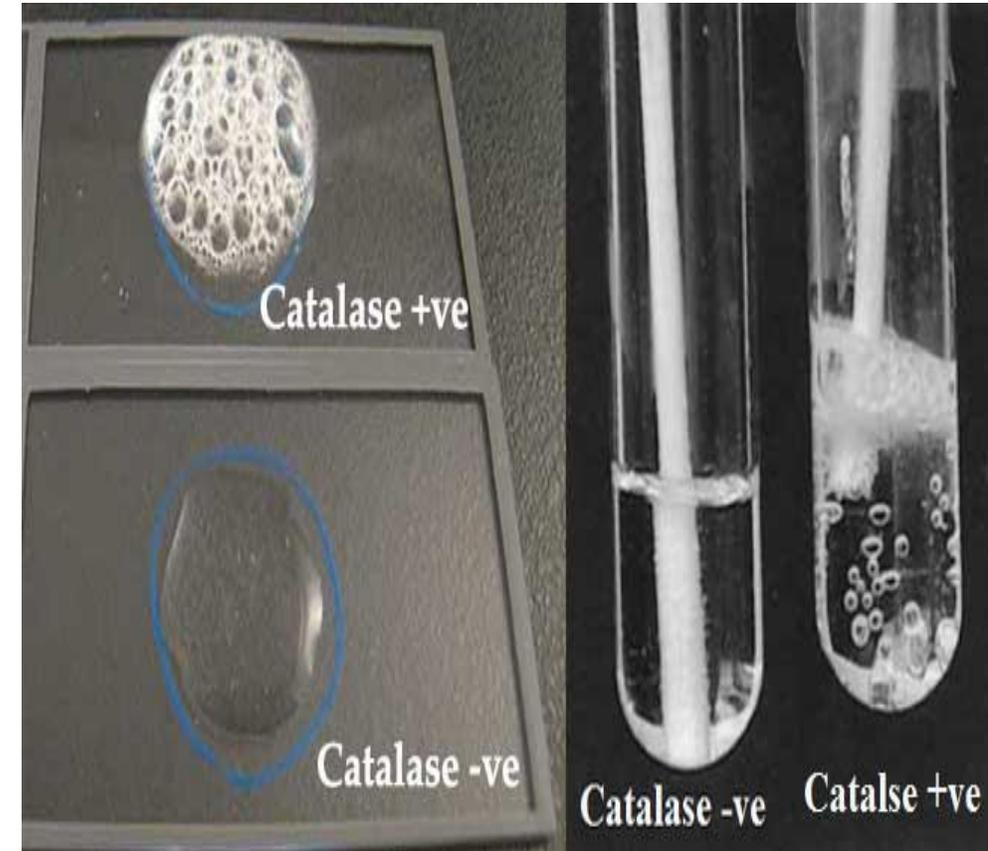
Biochemical Tests: Primary Biochemical Tests- Catalase test

Result:

A positive result is indicated by the rapid formation of bubbles

Clinical Use:

- Separates **Staphylococcus** (+) from **Streptococcus** (−)

Biochemical Tests: Primary Biochemical Tests- Coagulase test

Principle:

Coagulase is an enzyme that clots plasma (converts fibrinogen → fibrin)

Why Does *S. aureus* Produce Coagulase?

It's **a virulence factor** - helps the bacteria evade the immune system!

1. Coagulase clots plasma proteins around the bacteria → Creates a **fibrin shield** around bacterial cells

2. This shield protects bacteria from immune cells and antibodies

3. Bacteria can **multiply inside the fibrin clot** → Leads to localized infection→ **abscess formation**

Biochemical Tests: Primary Biochemical Tests- Coagulase test
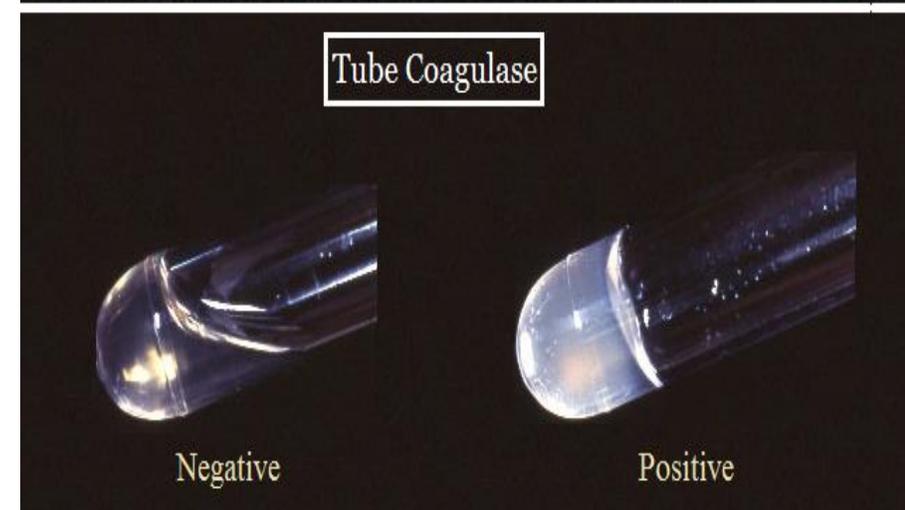
Purpose: Differentiates *Staphylococcus aureus* from other staphylococci

Two Methods:

- **Slide test (bound coagulase):** Clumping in 10 seconds

- **Tube test (free coagulase):** Clot formation in 4-24 hours (more reliable)

Result:

- **Positive:** *S. aureus* (pathogenic) ✓

- **Negative:** *S. epidermidis*, *S. saprophyticus* (less pathogenic) ✗
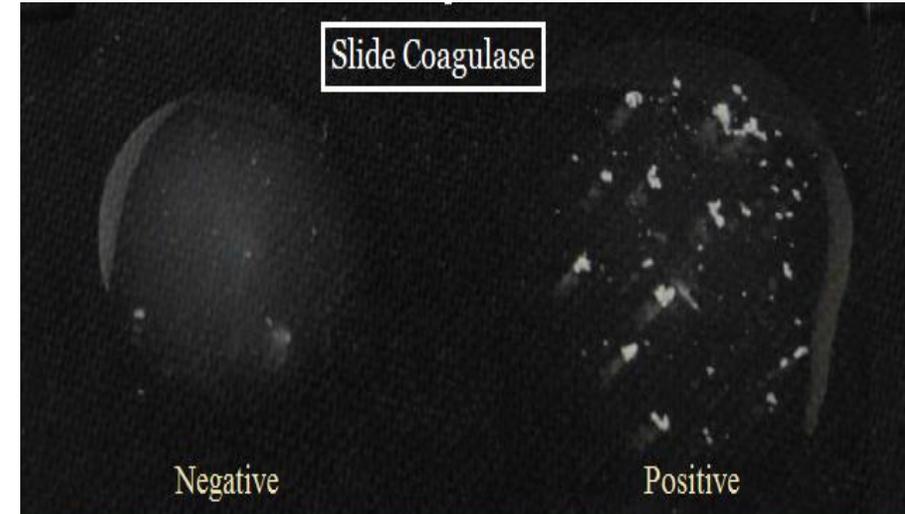
Biochemical Tests: Primary Biochemical Tests- Oxidase test

Principle: Detects **Cytochrome c oxidase,** the final enzyme in the aerobic electron transport chain

- It transfers electrons to oxygen (O_2) in aerobic respiration

Method:

- Apply bacterial colony to oxidase reagent disk/strip and observe within 10-30 seconds

- If the enzyme is present, it oxidizes the reagent → the Oxidized reagent changes from colorless → **purple/dark blue color**

Biochemical Tests: Primary Biochemical Tests- Oxidase test

Result:

- **Positive:** Purple/dark blue color ✓
- **Negative:** No color change ✗

Clinical Use:

- Differentiates **Enterobacteriaceae** (−) from other Gram-negative rods
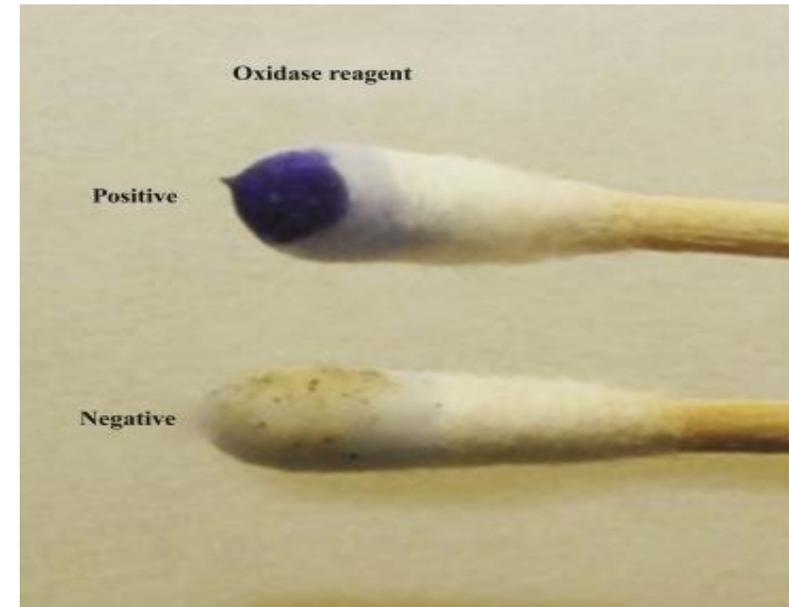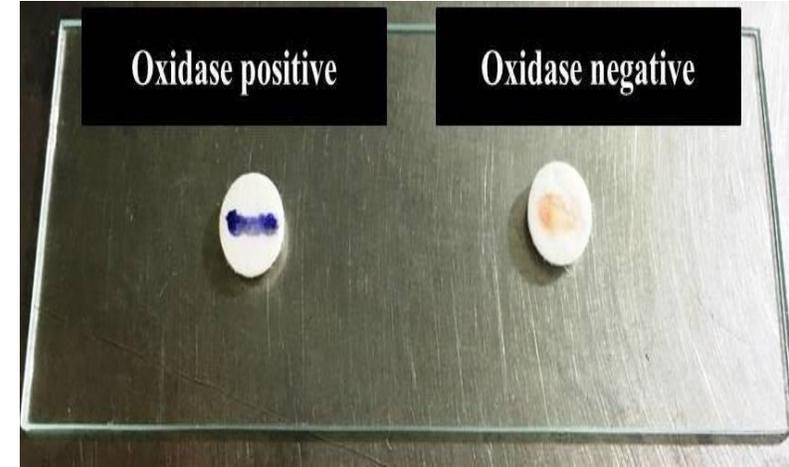
Biochemical Tests: Secondary Biochemical Tests

✓ Sugar fermentation tests

✓ Urease test

✓ H_2S (hydrogen sulphide) production test

Biochemical Tests: Secondary Biochemical Tests- Sugar fermentation tests

Purpose: Determine which sugars bacteria can ferment

Principle: The general fermentation formula:

Sugar (e.g., glucose) --[Bacterial enzymes]--$\rightarrow$ ATP? + Waste Products

Waste Products Can Include:

- **Organic acids** (lactic acid, acetic acid, formic acid, succinic acid)
- **Gases** (CO_2, H_2)
- **Alcohols** (ethanol)

Biochemical Tests: Secondary Biochemical Tests- Sugar fermentation tests

Principle:

• Different bacteria ferment different sugars

  • Common sugars tested: Glucose, Lactose, Sucrose, Mannitol, Maltose

Fermentation → Acid ± Gas

✓ Acid → pH indicator turns yellow

✓ Gas → trapped in Durham tube (small inverted tube)

negative carbohydrate fermentation

positive carbohydrate fermentation

fermentation and gas production

Carbohydrate ferm
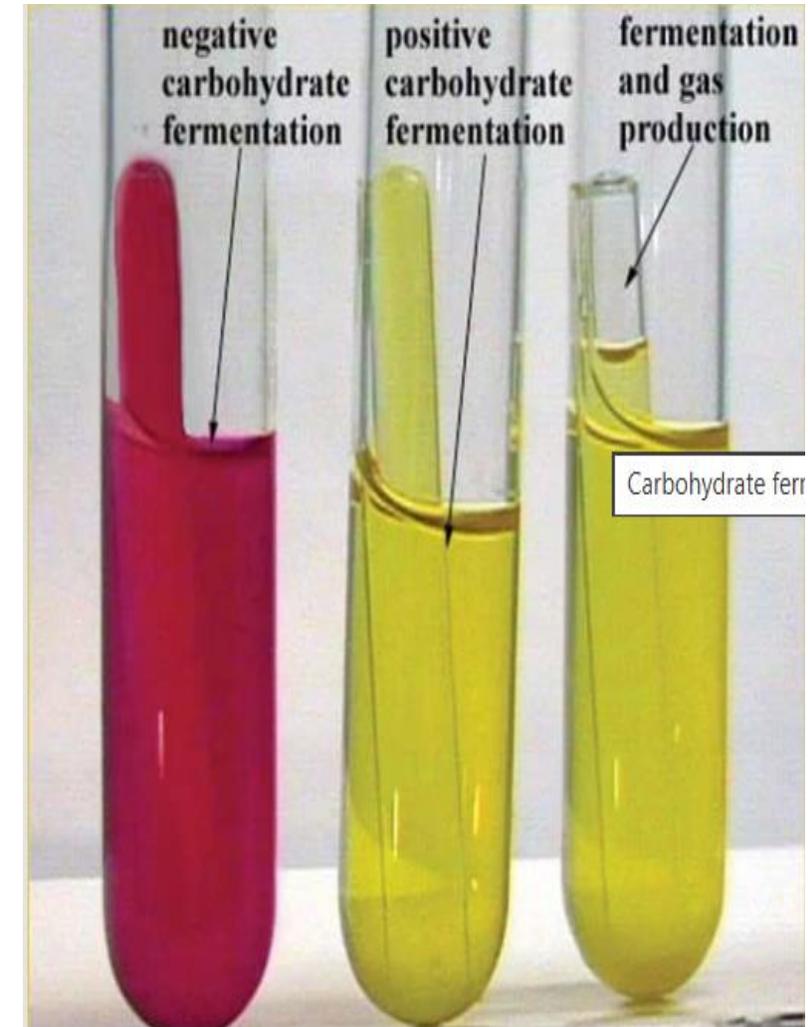
Biochemical Tests: Secondary Biochemical Tests- Sugar fermentation tests

Example: Clinical Scenario

Patient presents with **bloody diarrhoea**
→ Stool culture grows colourless colonies on MacConkey agar
(non-lactose fermenter)
→ Could be *Salmonella* or *Shigella* - both cause dysentery!
→ Need to differentiate them

Sugar Fermentation Pattern:

Bacteria	Glucose	Lactose	Mannitol	Gas Production
E. coli	+ (Yellow)	+ (Yellow)	+ (Yellow)	+ (Bubbles)
Salmonella	+ (Yellow)	− (Red)	+ (Yellow)	+ (Bubbles)
Shigella	+ (Yellow)	− (Red)	− (Red)	− (No bubbles)

Biochemical Tests: Secondary Biochemical Tests- Urease test

Purpose: Detects enzyme urease

Principle:

- Urease breaks down urea $\rightarrow$ ammonia + CO_2

- Ammonia raises pH $\rightarrow$ alkaline

- Phenol red indicator turns pink

- **Reaction:** Urea $\rightarrow$ $2NH_3$ + CO_2

Biochemical Tests: Secondary Biochemical Tests- Urease test

Result:

- **Positive:** Pink/magenta colour ✓

- **Negative:** Yellow/no change ✗

Clinical Use:

- Identifies *H. pylori* (causes gastric ulcers)

negative urea test

positive urea test
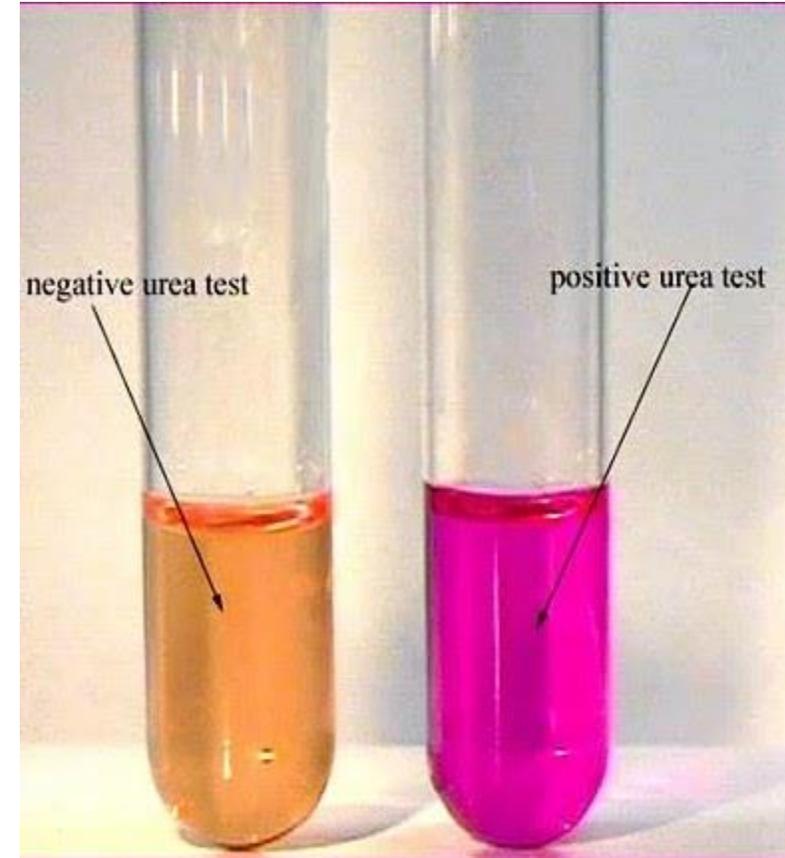
Biochemical Tests: Secondary Biochemical Tests- H$_2$S (hydrogen sulphide) production test

Purpose: Detects bacteria that produce H$_2$S gas

Principle:

- H$_2$S (Hydrogen Sulphide) = A gas produced when bacteria break down sulphur-containing amino acids (cysteine, methionine)

- H$_2$S reacts with iron salts → **black precipitate (FeS)**

Biochemical Tests: Secondary Biochemical Tests- H₂S (hydrogen sulphide) production test

Result:

- **Positive:** Black colour/precipitate ✓

- **Negative:** No black colour ✗

Clinical Use:

- ***Salmonella*** = strongly positive (important!)
- ***Proteus*** = positive
- ***E. coli, Shigella*** = negative

Why Important?

- Differentiates *Salmonella* from *Shigella* (both cause diarrhoea)

- Both are non-lactose fermenters, but *Salmonella* produces H₂S
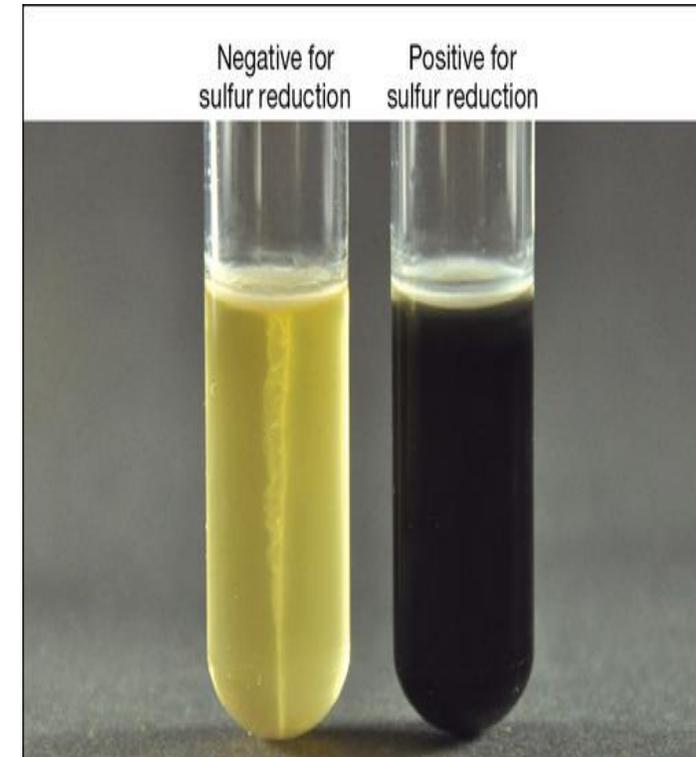
Biochemical Tests: Automated Identification System

Multiple biochemical tests in a small, convenient strip" – 20

- Each mini-tube contains dehydrated biochemical substrate
- Rehydrate with bacterial suspension → bacteria metabolize → colour changes
- Read results → convert to numerical code → look up in database

Biochemical Tests: Automated Identification System- Example: API Staph

Contains 20 tests including:

- Sugar fermentation (glucose, mannose, mannitol, etc.)

- Enzyme tests (urease)

Result Example:

Visual pattern → Code: 6736153 → Database lookup → **S. aureus** (97.8% confidence)

Gram-positive

Cocci

Rods

Catalase test

⊖ *Streptococci*

⊕ *Staphylococci*

Growth on sheep's blood agar

Coagulase test

⊖ *S. saprophyticus*
S. epidermidis

⊕ *S. aureus*

None

γ-hemolytic

β-hemolytic

α-hemolytic

Enterococcus

Group A
S. pyogenes

Group B
S. agalactiae

Capsule
S. pneumoniae

No Capsule
Viridans streptococci

Gram-negative bacteria

- **Diplococci**
  - Aerobic
    - **Maltose utilization**
      - No
        - *N gonorrhoeae*
        - *Moraxella*
      - Yes
        - *N meningitidis*

- **Coccobacilli**
  - *H influenzae*
  - *B pertussis*
  - *Pasteurella*
  - *Brucella*
  - *F tularensis*

- **Bacilli**
  - **Lactose fermenting**
    - No
      - **Oxidase-positive**
        - No
          - **H₂S-producing**
            - No
              - *Shigella*
              - *Yersinia*
            - Yes
              - *Salmonella*
              - *Proteus*
        - Yes
          - *Pseudomonas*
    - Yes
      - Slow
        - *Citrobacter*
        - *Serratia*
      - Fast
        - **Indole-positive**
          - No
            - *Klebsiella*
            - *Enterobacter*
          - Yes
            - *E coli*

- **Spiral, comma, or S-shaped**
  - Oxidase-positive
    - Grows in 42 C
      - *C jejuni*
    - Grows in alkaline media
      - *V cholerae*
    - Urease producing
      - *H pylori*

Key Message:

"Accurate bacterial identification is the foundation of effective patient care"

Feedback Form

أطباء وطبيبات دفعة سنة ثانية الكرام

يرجى التكرم بكتابة نقد بناء <u>دون مدح أو شكر</u>

ما هي المشاكل والعقبات التي واجهتموها معي أثناء التدريس وما هي طريقة التطوير والتحسين بوجهة نظرك؟

بإمكانك بتكتب اي شيء بيخطر على بالك، مهما كان

<u>ملاحظة:</u> النموذج هذا تم تصميمه بحيث ما يظهر اسماء المشاركين. بتقدر تكتب وانت مرتاح مهما كانت ملاحظتك

د. حلا الطراونة

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