Biochemical tests for Bacterial Identification and Classification

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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

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

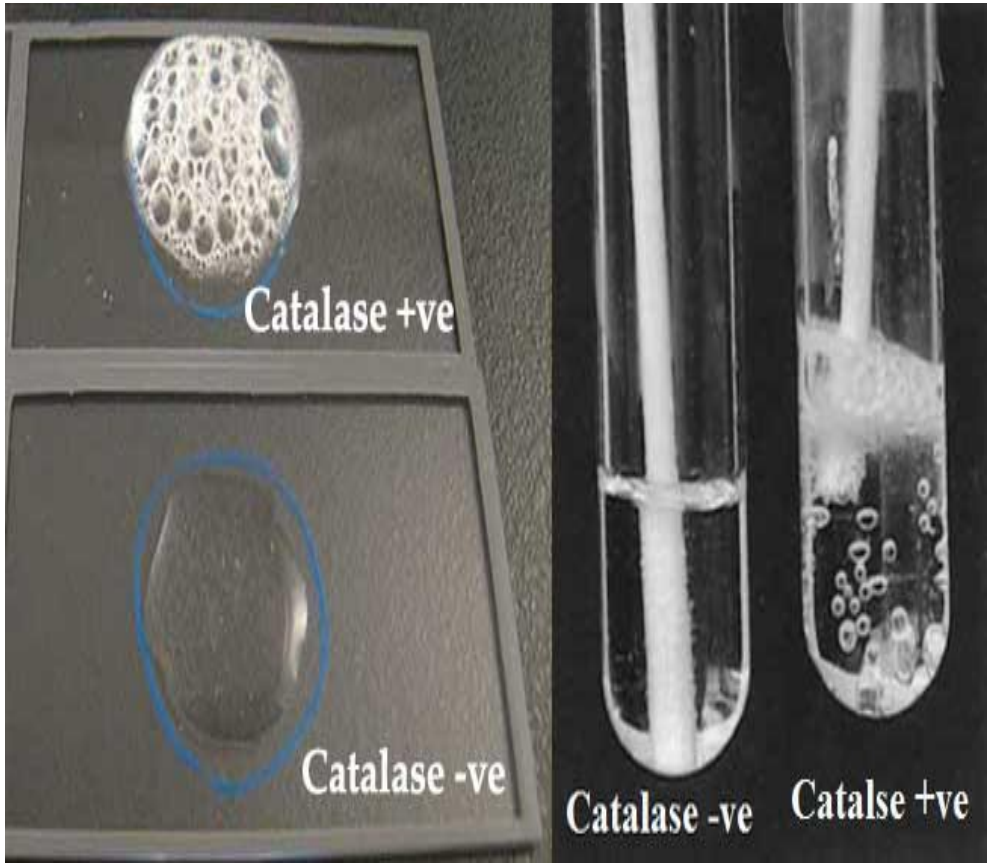
Biochemical Tests: Primary Biochemical Tests- Catalase test

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

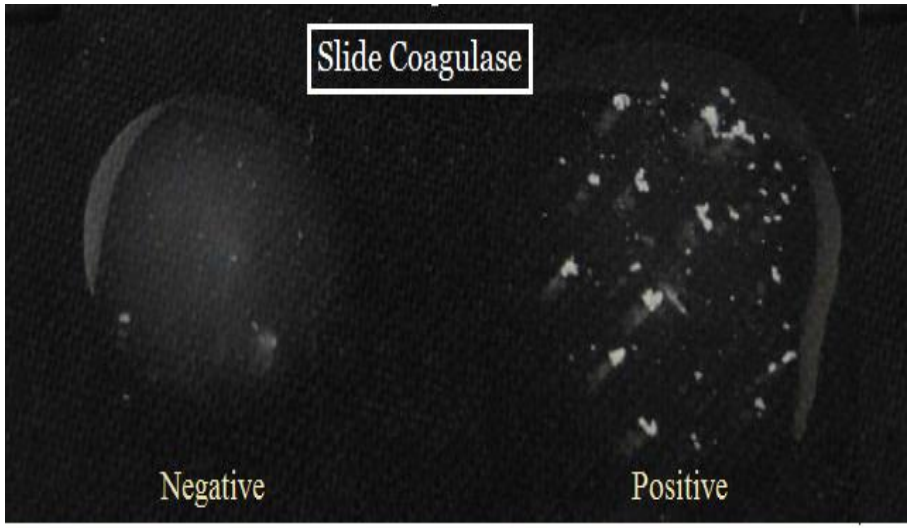
Purpose: Differentiates *Staphylococcus aureus* from other staphylococci

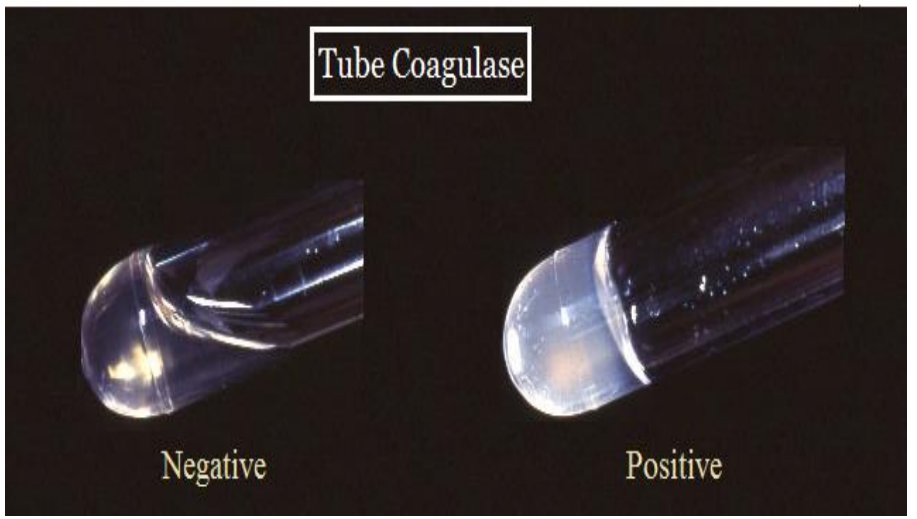
Two Methods:

- **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

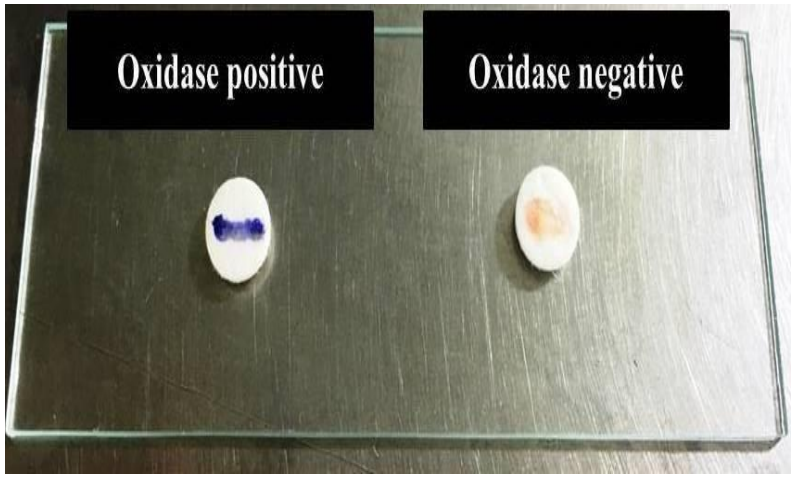
Principle: Detects **Cytochrome C oxidase,** the final enzyme in the aerobic electron transport chain

- 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

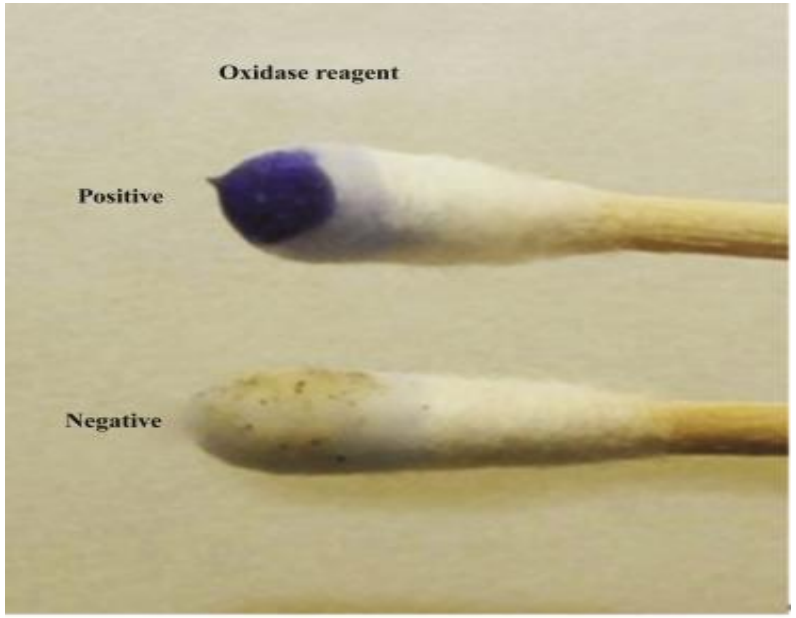


Result:

- **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 **!**
- ✓ Voges-proskauer (vp) test*
- ✓ Urease test
- ✓ Citrate Utilization test*
- ✓ H_2S (hydrogen sulphide) production test
- ✓ Indole test*

Biochemical Tests: Secondary Biochemical Tests- Sugar fermentation tests

Purpose: Determine which sugars bacteria can ferment

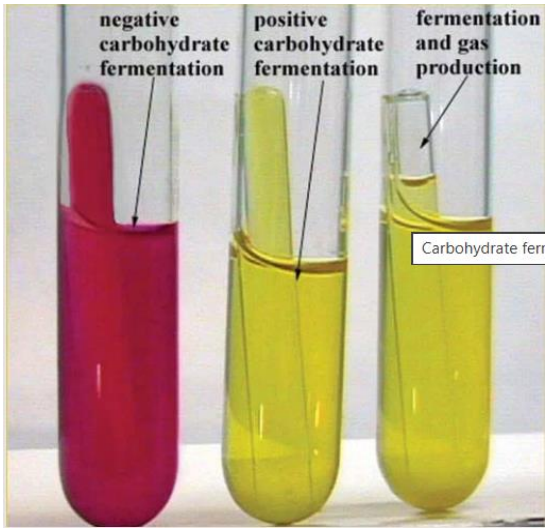
Principle: The general fermentation formula:

Sugar (e.g., glucose) --[Bacterial enzymes]--→ 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**

✓If No acid production (negative test) →

✓ red (phenol red) or blue (Bromothymol Blue)

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

Biochemical Tests: Secondary Biochemical Tests- Voges-proskauer (vp) test*

Principle: Detects acetoin production from glucose fermentation (butanediol pathway)

The Detection Chemistry:

Acetoin + α-naphthol + KOH + O_2 → Diacetyl + Creatine (from KOH reagent) → RED COMPLEX

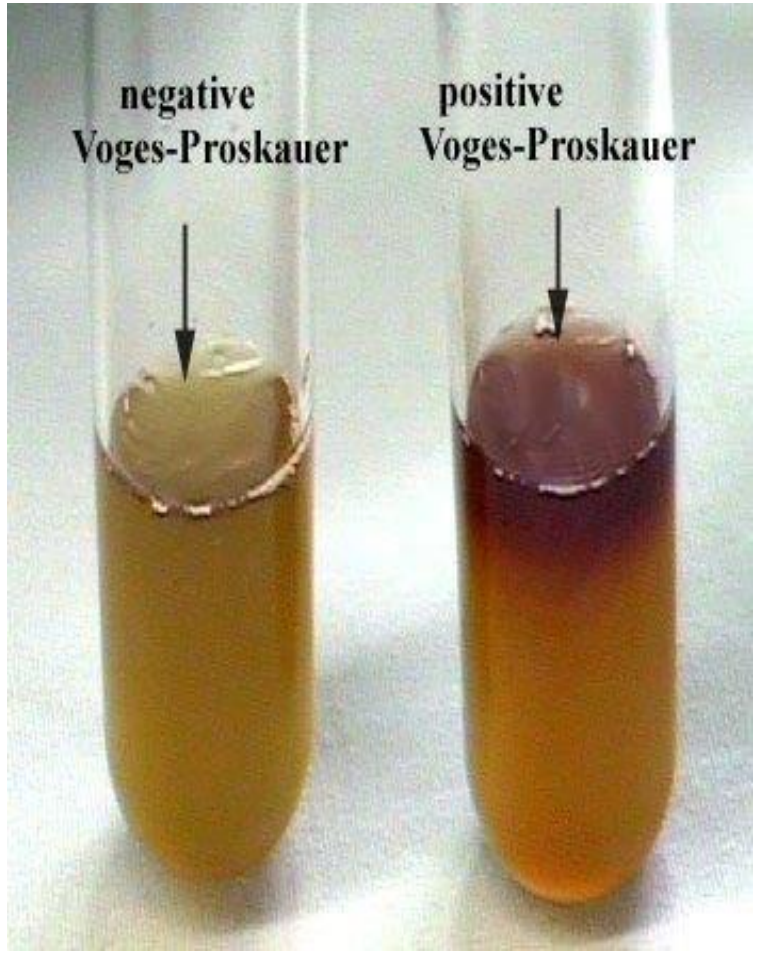
Procedure:

- Inoculate VP broth and incubate 48 hours at 37°C
- Transfer 1 ml to clean tube and add reagent A (α-naphthol) then add reagent B (KOH)
- Wait 15-30 minutes and observe red complex formation.

Biochemical Tests: Secondary Biochemical Tests- Voges-proskauer (vp) test*

Results:

- **POSITIVE:** Red/pink colour → Klebsiella, Enterobacter
- **NEGATIVE:** No colour change → *E. coli*, Salmonella



Biochemical Tests: Secondary Biochemical Tests- Urease test

Purpose: Detects enzyme urease

Principle:

- Urease breaks down urea → ammonia + CO_2
- Ammonia raises pH → alkaline
- Phenol red indicator turns pink
- **Reaction:** Urea → $2NH_3$ + CO_2

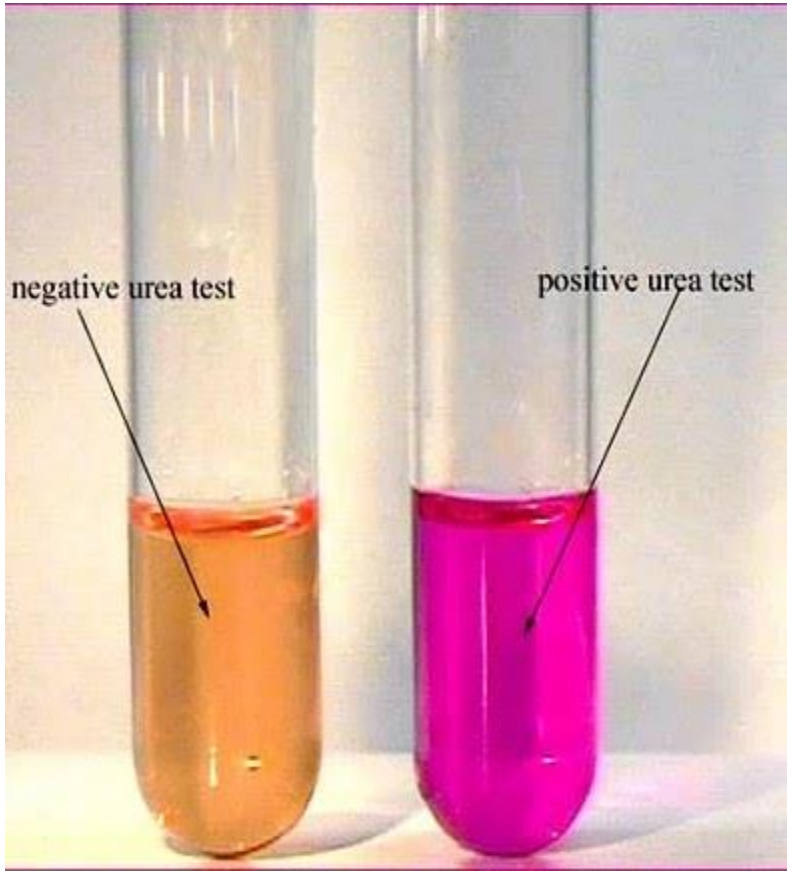
Biochemical Tests: Secondary Biochemical Tests- Urease test

Result:

- **Positive:** Pink/magenta colour ✓
- **Negative:** Yellow/no change ✗

Clinical Use:

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



Biochemical Tests: Secondary Biochemical Tests- Citrate utilization test*

Principle: Tests if bacteria can use citrate as sole carbon source and grow

Procedure:

- Lightly streak Simmons citrate agar slant (green medium)
- Incubate 24-48 hours at 37°C with loose cap
- Observe for colour change and growth

Biochemical Tests: Secondary Biochemical Tests- Citrate utilization test*

Results:

- **POSITIVE:** Green → Blue with growth → Klebsiella, Enterobacter, Citrobacter
- **NEGATIVE:** Remains green, no growth → *E. coli*, Shigella

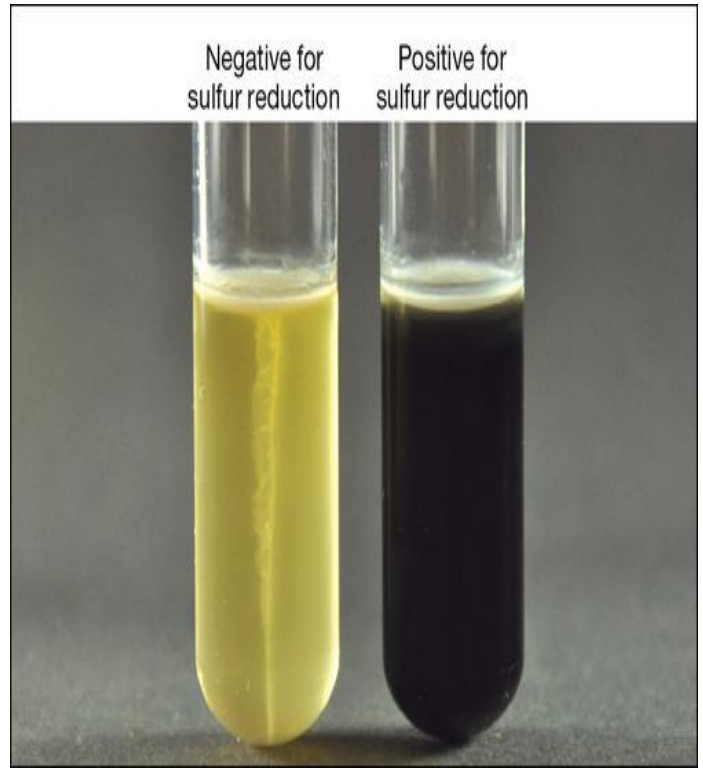
Biochemical Tests: Secondary Biochemical Tests- H_2S (hydrogen sulphide) production test

Purpose: Detects bacteria that produce H_2S gas

Principle:

- H_2S (Hydrogen Sulphide) = A gas produced when bacteria break down sulphur-containing amino acids (cysteine, methionine)
- H_2S reacts with iron salts → **black precipitate (FeS)**

Biochemical Tests: Secondary Biochemical Tests- H_2S (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 H_2S

Biochemical Tests: Secondary Biochemical Tests- Indole test*

Principle: Tests if bacteria can break down tryptophan (amino acid) into indole using tryptophanase enzyme

Tryptophan + Water → **Indole** + Pyruvic acid + Ammonia

Procedure:

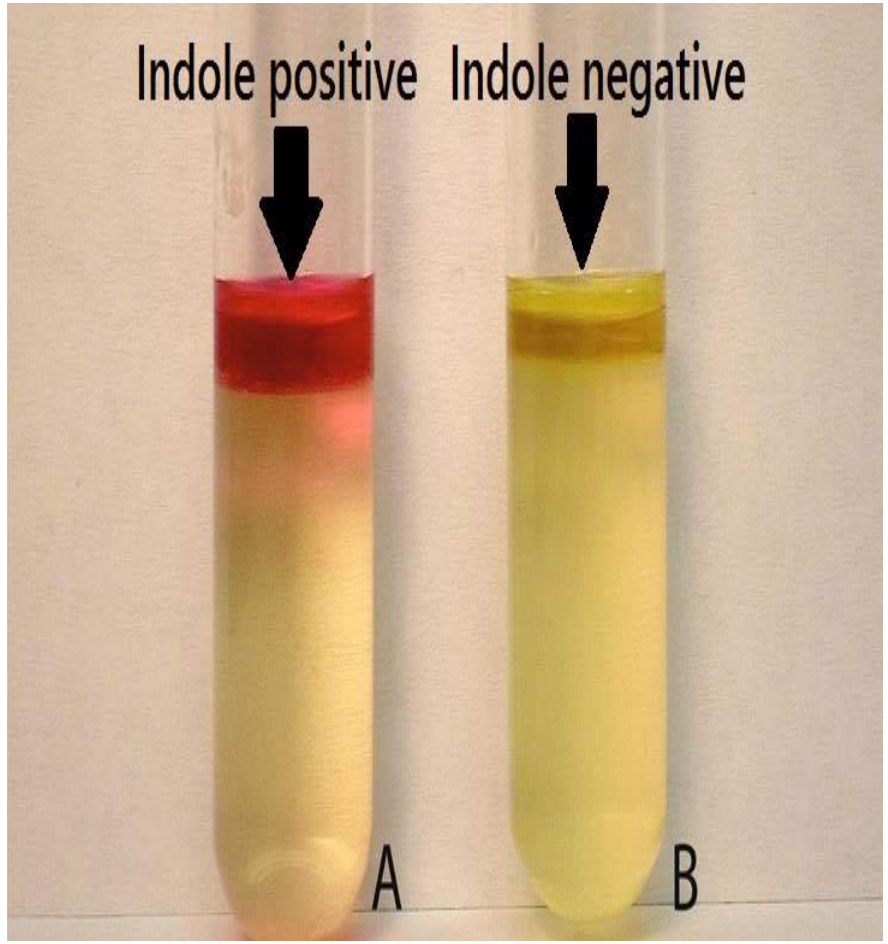
- Inoculate tryptophan broth with test organism
- Incubate 24-48 hours at 37°C
- Add 5 drops Kovac's reagent (p-dimethylaminobenzaldehyde), Read immediately

Indole + Kovac's reagent (in acid) → Rosindole (RED!)

Biochemical Tests: Secondary Biochemical Tests- Indole test*

Results:

- **POSITIVE:** Cherry red ring at surface → *E. coli, Proteus vulgaris*
- **NEGATIVE:** Yellow or no colour → Klebsiella, Enterobacter



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)

pH Indicators in Microbiology

Key Points:

- All detect pH changes from bacterial metabolism
- Choose based on what pH range you need to detect

1. **Phenol Red** → Best for **acid** from sugar fermentation (most common)
2. **Bromothymol Blue** → Best for detecting **alkaline shifts** (citrate test)
3. **Methyl Red** → Detects strong acid production (MR test for mixed acid fermentation)

For your own knowledge; **not for exams**

pH Indicators in Microbiology

Indicator	pH Range	Acidic Color	Neutral Color	Alkaline Color	Common Use
Methyl Red	4.4 - 6.2	**RED**	**Orange**	**YELLOW**	Mixed acid fermentation test
Phenol Red	6.8 - 8.4	**YELLOW**	**RED**	**Pink**	Sugar fermentation tubes, TSI agar
Bromothymol Blue	6.0 - 7.6	**YELLOW**	**GREEN**	**BLUE**	Citrate test

For your own knowledge; **not for exams**


Thank you!