MEDICAL MICROBIOLOGY BIOCHEMICAL TEST 3

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BIOCHEMICAL TESTS

- Group of tests, used to differentiate between bacterial genera/species.
- **Biochemical tests classified as:**
  1. Enzymatic tests
  2. Metabolic pathways tests
     a) Carbohydrate oxidation and fermentation tests
     b) Amino acids degradation tests
     c) Single substrate utilization tests
  3. Antibiotic inhibition tests
  4. Specific tests

METABOLIC PATHWAYS TESTING

3) Establishing Inhibitor Profiles

1. Bacitracin susceptibility test
2. Bacitracin and sulfamethoxazole-trimethoprim susceptibility test
3. Novobiocin susceptibility test
4. Vancomycin susceptibility test
5. Antibiotic disks for presumptive identification of anaerobes

4) Other more specific tests

1. Growth in various NaCl concentrations
2. Susceptibility to optochin and solubility in bile
3. Ability to hydrolyze esculin in the presence of
4. CAMP

BACITRACIN SUSCEPTIBILITY TESTING

- To differentiate *Micrococcus* and *Stomatococcus* from *Staphylococcus* when combined with other procedures such as the modified oxidase test.

Principle

- Bacitracin (0.04 units) inhibits the growth of *Micrococcus* and *Stomatococcus* and Group A *Streptococcus* while having no effect on Staphylococcus which is resistant.

Results

- Susceptible (S): zones of inhibition ≥ 10 mm.
- Resistant (R): zones of inhibition ≤ 9 mm.

BACITRACIN AND SULFAMETHOXAZOLE-TRIMETHOPRIM SUSCEPTIBILITY TEST

- To identify the different species of *Streptococcus* especially Group A and Group B beta hemolytic streptococci.

Principle:

- Group A beta hemolytic streptococci (*Streptococcus pyogenes*) are susceptible to 0.04 units bacitracin but resistant to 1.25 ug sulfamethoxazole-trimethoprim (SXT).
- Group B beta hemolytic streptococci (Streptococcus agalactiae) resistant to both bacitracin and SXT.

Results

- **Susceptible (S):** any zone of inhibition around either disk.
- **Resistant (R):** growth up to the disk (no zone of inhibition).

INTERPRETATION

Streptococcus spp.	Bacitracin	SXT
Group A	Susceptible	Resistant
Group B	Resistant	Resistant
Group C, F, G	Resistant	Susceptible

NOVOBIOCIN SUSCEPTIBILITY TESTING

- To differentiate the different species of coagulase negative staphylococci.

Principle:

- After incubation with 5 ug of novobiocin, *Staphylococcus saprophyticus* is not inhibited (resistant) by the antibiotic whereas *Staphylococcus epidermidis* are susceptible to novobiocin.

Results:

- Susceptible (S): zone greater than 16 mm
- Resistant (R): zone diameter ≤ 16 mm

VANCOMYCIN SUSCEPTIBILITY TEST

- To differentiate Pediococcus from other alpha hemolytic streptococcus.

Principle:

- After incubation with 5 ug of vancomycin, Pediococcus is not inhibited (resistant) by the antibiotic whereas Viridans streptococcus is susceptible to vancomycin.

Results:

- **Susceptible:** zone of inhibition
- **Resistant:** no zone of inhibition

ANTIBIOTIC DISKS FOR PRESUMPTIVE IDENTIFICATION OF ANAEROBES

- To determine an anaerobe's inhibition that can be used for presumptive identification based on its characteristic susceptibility pattern to colistin (10 ug), vancomycin (5 ug) and kanamycin (1 mg).

Principle:

- Most anaerobes have a characteristic susceptibility pattern to the above mentioned antibiotics.

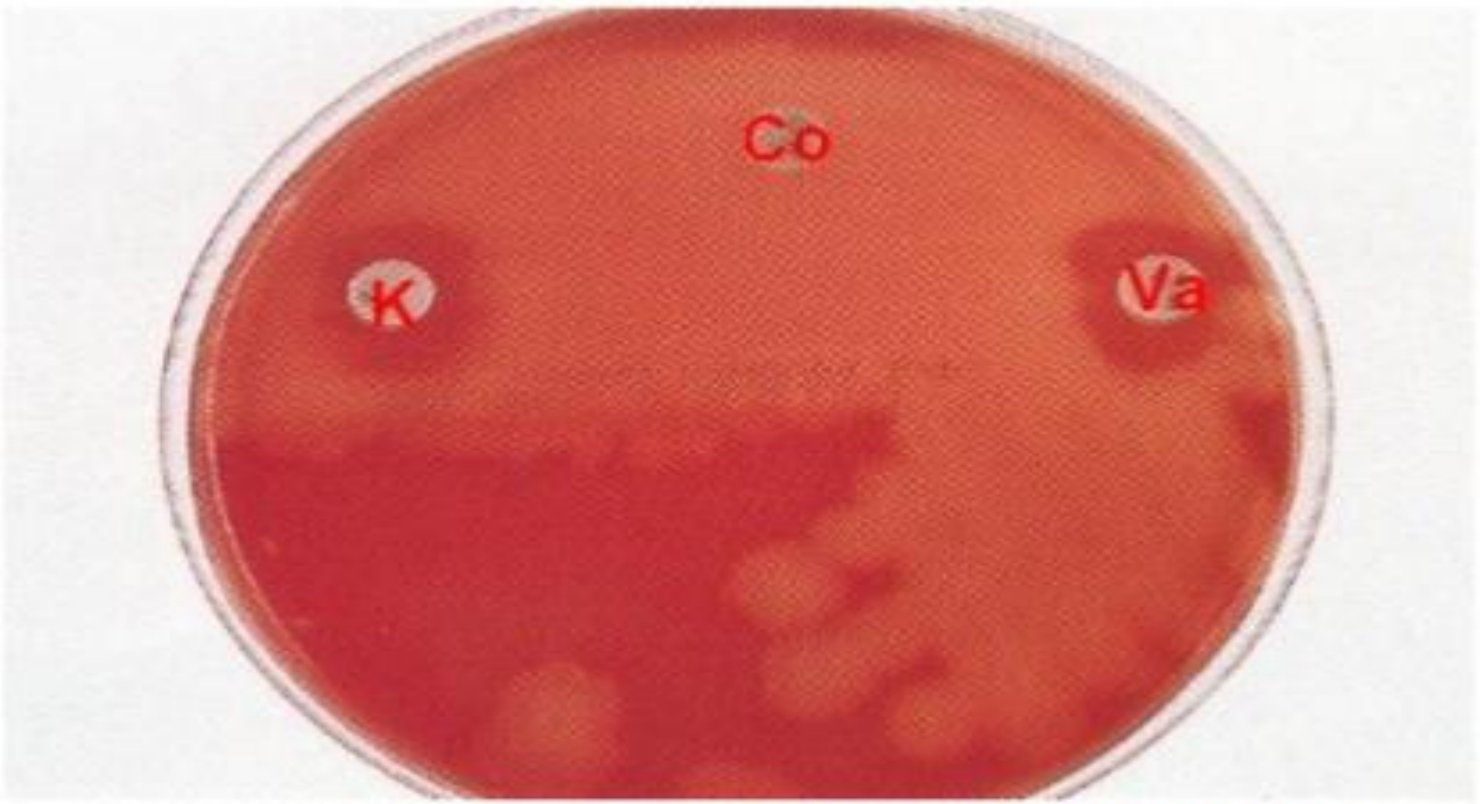
1. Kanamycin: inhibits facultative gram (– ve) bacilli.
2. Vancomycin: inhibits facultative & obligate gram (+ve) bacteria
3. Colistin: inhibits facultative gram (– ve) bacilli.

10-7 Special potency antimicrobial disks for presumptive identification of anaerobes. The pattern of susceptibility to the three antibiotic disks: kanamycin (1000 µg), colistin (10 µg), and vancomycin (5 µg) can help differentiate among anaerobic genera using these criteria:

Microorganism type	Kanamycin	Vancomycin	Colistin
Clostridium	S (1, 2)	S	R
Bacteroides fragilis group	R (1)	R	R
Bacteroides ureolyticus group	S	R	S
Fusobacterium species	S	R	S
Veillonella species	S	R	S
Porphyromonas species	R	S	R
Prevotella species	R	R	V
Peptostreptococcus anaerobius	R (3)	S	R
Other gram-positive cocci	S	S	R

(1) Some strains are kanomycin-resistant
(2) V, variable; R, resistant; S, susceptible
(3) Rare strains are susceptible

RESULTS



Susceptible (S): zone greater than 10 mm

Resistant (R): zone of 10 mm or less

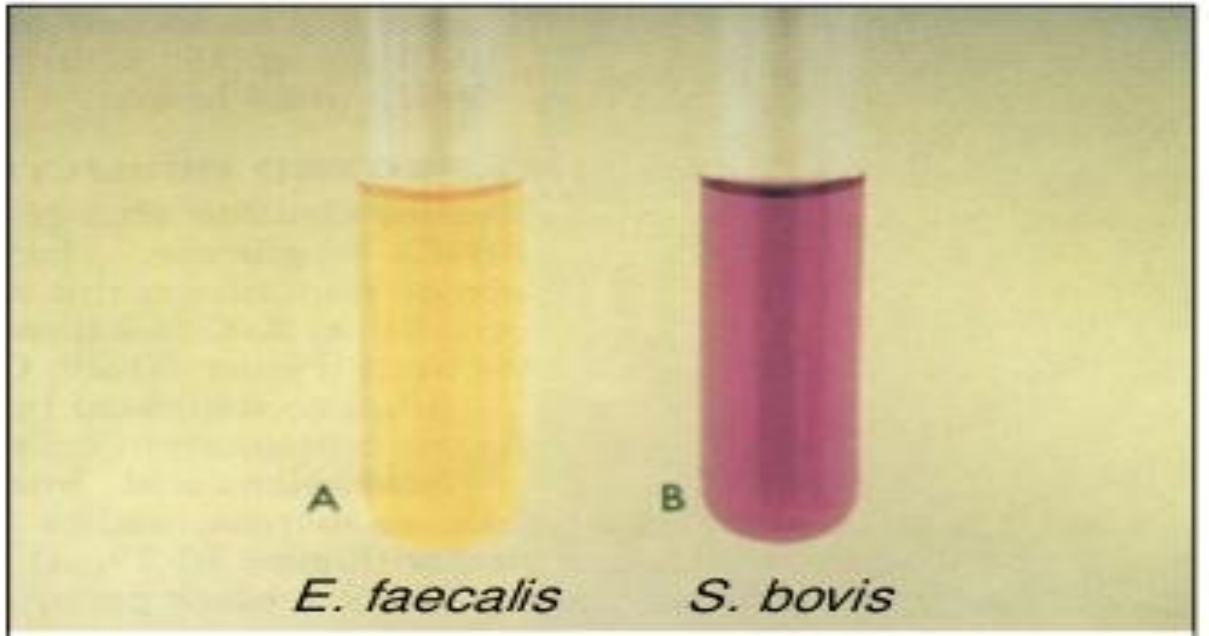
SALT TOLERANCE (6.5% NACL) TEST

- To classify bacteria based on their ability to grow in the presence of 6.5% NaCl, a characteristic of certain species of gram positive and gram negative bacilli.
- To differentiate the Group D (salt tolerant) from the nonenterococci (intolerant).
- **Results:**

	Growth in nutrient broth	Growth in 6.5% NaCl
Positive	Equal	Equal
Negative	Good growth	Very weak growth

RESULTS AND INTERPRETATION

- **Positive:** if growth is equivalent in both media (salt tolerant)
- **Negative:** growth on the salt containing medium is very weak or absent & growth in the salt free medium is good (intolerant).



A) Positive B) negative

- **Indicator:** bromcresol purple, when positive the medium turns yellow from purple or the appearance of growth.

GROWTH IN BILE ESCULIN AGAR

- To distinguish Group D streptococci and Enterococcus species from other Lancefield group of streptococci.

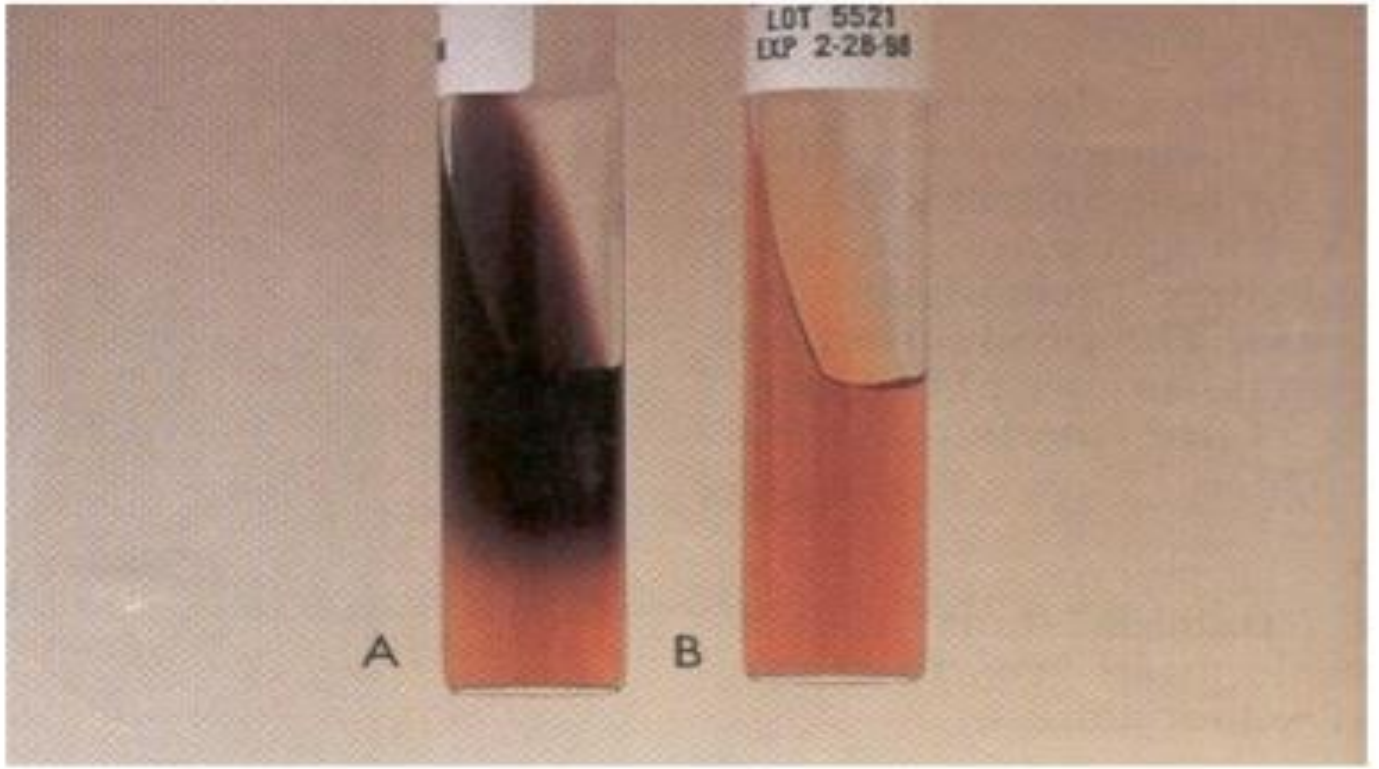
Principle:

- Based on the organisms ability to grow in 40% bile and to hydrolyze esculin to produce esculetin.
- Esculin reacts with ferric citrate to form a brown black precipitate.

Results:

- Positive: growth indicates tolerance to grow in 40% bile, **blackening** indicates hydrolysis of esculin.
- Negative: lack of growth indicates inability to grow in 40% bile, no color change indicates inability to hydrolyze esculin

RESULTS AND INTERPRETATION



A. Positive: *Enterococcus faecalis*
B. Negative: *Streptococcus viridans*

OPTOCHIN SUSCEPTIBILITY TESTING

- To differentiate Streptococcus pneumoniae from other alpha hemolytic streptococci.

Principle:

- In the presence of optochin, colonies of *Streptococcus pneumoniae* are selectively lysed indicated by a zone of inhibition after incubation under increased CO_2.
- Other alpha hemolytic streptococci are resistant to optochin.

RESULTS AND INTERPRETATION

- Positive: zone of inhibition at least 14 mm in diameter using (10 µg, P disk) and at least 10 mm when using (6 µg, P disk).
- Negative: growth up to the disk or a zone of inhibition less than 14 mm with (10, µg, P disk) or less than 10 mm with (6 µg, P disk).

A. Positive – Streptococcus pneumoniae
B. Negative – Viridans streptococci

BILE SOLUBILITY TEST

- To differentiate *Streptococcus pneumoniae* (which is positive for bile solubility) from other alpha hemolytic streptococci.

Principle:

- Pneumococcal colonies are rapidly lysed by bile or a solution of a bile salt such as sodium deoxycholate.
- Lysis depends on the presence of an intracellular autolytic enzyme.
- Bile salts lower the surface tension between the bacterial cell membrane and the medium thus accelerating the organism's natural autolytic process

RESULTS

- **Positive:** colony disintegrates; an imprint of the lysed colony may remain within the zone.
- **Negative:** intact colonies.

CAMP TEST

- To demonstrate the phenomena of synergistic hemolysis between group B streptococcus and beta hemolytic *Staphylococcus aureus*.

Principle:

- A characteristic "arrowhead" hemolytic pattern results when the organism is streaked perpendicular to beta hemolytic *Staphylococcus aureus*.

RESULTS AND INTERPRETATION

- **Positive:** a zone of enhanced hemolysis given by an arrowhead appearance at the junction of the *Staphylococcus* and *Streptococcus*, indicative of Group B streptococcus.
- **Negative:** no zone of enhanced hemolysis not indicative of Group B streptococci.