

Bio 51 Student Reference Guide

Prepared for Kingsborough Learning Center

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Identification of Bacteria Associated with the Throat infection

The possible bacteria:

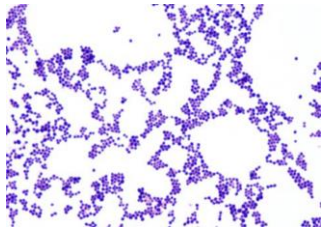
Staphylococcus aureus or *Staphylococcus epidermidis*.

1. Gram reaction and shape:

Observation: Purple, round in clusters

Result and explanation: Gram-positive staphylococci. The bacteria's cell wall is made of a thick peptidoglycan layer. The decolorizing agent (alcohol) does not wash off crystal violet from Gram-positive bacteria, the cell wall remains purple.

Organisms indicated: *Staphylococcus aureus* or *Staphylococcus epidermidis*



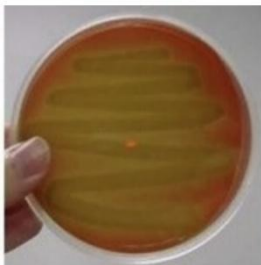
<https://pl.pinterest.com/pin/381680137149195988/>

2. Hemolysis (blood agar):

Observation: White bacteria growth on the red color agar

Result and explanation: Gamma or nonhemolytic. Bacteria have no hemolysins to lyse the red blood cells. If hemolysins are present, the clearing area around the growth is visible.

Organisms indicated: *Staphylococcus epidermidis*



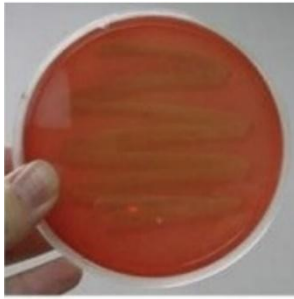
Alpha Hemolysis

Alpha hemolysis – partial or green breakdown of red blood cells



Beta Hemolysis

Beta hemolysis – complete breakdown of red blood cells



Gamma Hemolysis

Gamma hemolysis – no breakdown of red blood cells

<https://microbiologyinfo.com/haemolysis-of-streptococci-and-its-types-with-examples/>

3. Mannitol salt agar (MSA):

Observation: Abundant growth, agar is red

Result and explanation: Halotolerant staphylococci are negative for mannitol fermentation. The medium is selective and differential. High salt concentration selects for *Staphylococcus*. Also, the medium contains sugar mannitol and pH indicator phenol red. If mannitol was fermented, the phenol red indicator causes the medium to turn yellow.

Organisms indicated: *Staphylococcus epidermidis*



mannitol fermentation positive – yellow

mannitol fermentation negative - red

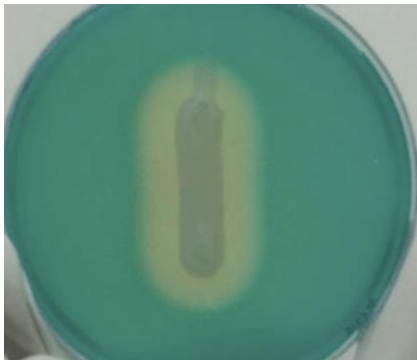
http://www.journaldmims.com/viewimage.asp?img=JDattaMegheInstMedSciUniv_2018_13_2_91_246006_f1.jpg

4. DNase:

Observation: Growth is surrounded by cloudy agar

Result and explanation: No DNase present. DNase agar is a differential medium that indicates if bacteria produce an enzyme DNase to hydrolyze DNA. Methyl green is an indicator that binds to negatively charged DNA. When DNase is present, the DNA is broken down and does not bond to methyl green. The growth is surrounded by a clear area. The cloudy agar indicates that no DNA was broken down.

Organisms indicated: *Staphylococcus epidermidis*



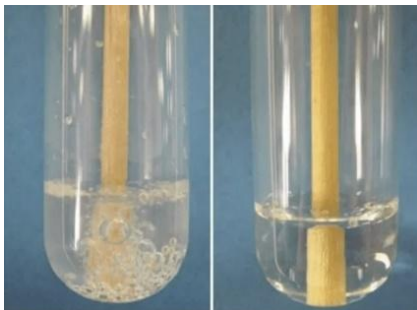
https://www.researchgate.net/figure/DNase-production-by-S-marcescens-in-DNA-agar-medium_fig2_281225603

5. Catalase:

Observation: Bubbles are visible after hydrogen peroxide addition

Result and explanation: Catalase positive. Bubbles indicate that catalase converts hydrogen peroxide to oxygen. No bubbles would indicate that hydrogen peroxide was not converted to oxygen.

Organisms indicated: *Staphylococcus aureus* or *Staphylococcus epidermidis*



catalase positive – bubbles

catalase negative – no bubbles

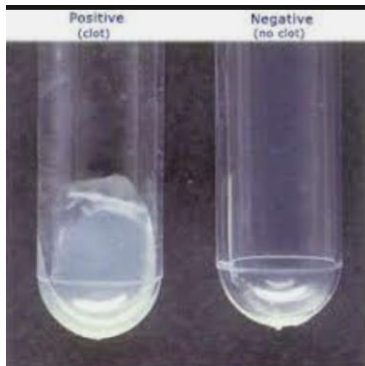
<https://microbeonline.com/catalase-test-principle-uses-procedure-results/>

6.Coagulase:

Observation: Only liquid in the test tube

Result and explanation: Negative for coagulase. Liquid and no clotting or clumping in the test tube indicates that there is no enzyme coagulase that causes the proteins in the blood to form clots. In the case of clots formation, the result is positive for coagulase.

Organisms indicated: *Staphylococcus epidermidis*



<https://microbiologyinfo.com/coagulase-test-principal-procedure-types-interpretation-and-examples/>

Identification of Bacteria Associated with the Gastrointestinal infection

The possible bacteria:

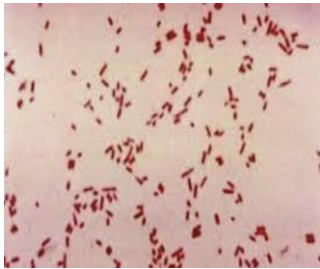
Enterococcus faecalis, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, or *Staphylococcus aureus*.

7. Gram reaction and shape:

Observation: Pink rods scattered around the field

Result and explanation: Gram-negative bacilli, individual. The bacteria's cell wall is made of a thin peptidoglycan layer covered by an outer plasma membrane. The decolorizing agent (alcohol) washes off crystal violet from Gram-negative bacteria, which became colorless, the counterstain, safranin, dyes the cell wall to pink color.

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*



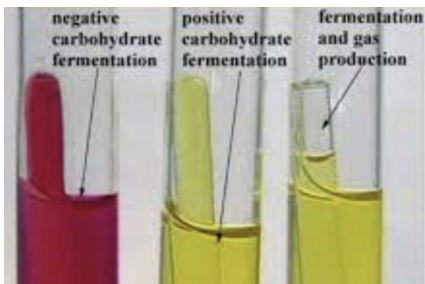
<https://microbenotes.com/escherichia-coli-e-coli/>

8. Glucose Durham Tube Fermentation:

Observation: Broth is yellow, a bubble in the Durham tube

Results and explanation: Positive for glucose fermentation and positive for gas production. Glucose fermentation produces an acidic environment, and the pH indicator phenol red turns yellow in an acidic environment. A bubble forms in a Durham tube due to CO₂ gas production

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*



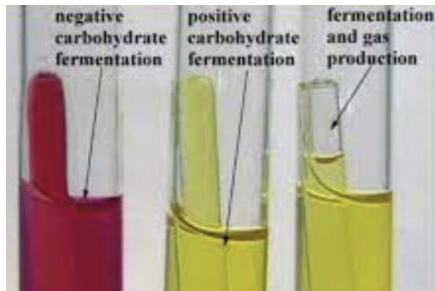
<https://microbiologynote.com/carbohydrate-fermentation-test-sugar-fermentation-test/>

9. Lactose Durham Tube Fermentation:

Observation: Broth is yellow, a bubble in the Durham tube

Results and explanation: Positive for lactose fermentation and positive for gas production. Lactose fermentation produces an acidic environment, and the pH indicator phenol red turns yellow in an acidic environment. A bubble forms in a Durham tube due to CO₂ gas production

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*



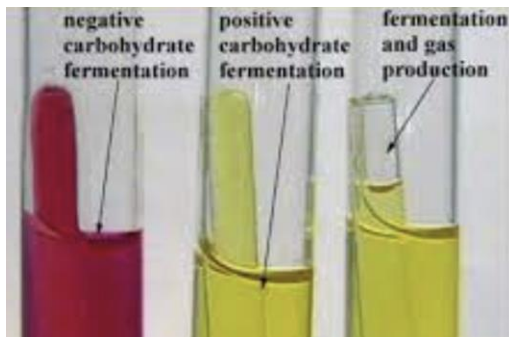
<https://microbiologynote.com/carbohydrate-fermentation-test-sugar-fermentation-test/>

10. Mannitol Durham Tube Fermentation:

Observation: Broth is yellow, a bubble in the Durham tube

Result and explanation: Positive for mannitol fermentation and positive for gas production. Mannitol fermentation produces an acidic environment, and the pH indicator phenol red turns yellow in an acidic environment. A bubble forms in a Durham tube due to CO₂ gas production

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*



<https://microbiologynote.com/carbohydrate-fermentation-test-sugar-fermentation-test/>

11. Indole production:

Observation: No red ring is visible after Kovac's reagent addition

Result and explanation: Indole negative. Tryptophan broth is used as a medium. If the bacteria contain an enzyme tryptophanase, then tryptophan is hydrolyzed to produce indole, pyruvic acid, and ammonia. Added Kovac's reagent reacts with indole, forming a red ring. Kovac's reagent does not react with indole, and no red ring is formed.

Organisms indicated: *Enterobacter aerogenes*, *Salmonella typhimurium*, *Enterococcus faecalis*, or *Staphylococcus aureus*.



indole positive – red ring

indole negative – no red ring

<https://microbiologyinfo.com/indole-test-principle-reagents-procedure-result-interpretation-and-limitations/>

12. Methyl Red:

Observation: Broth is yellow after the addition of Methyl red

Result and explanation: Negative for methyl red. Glucose was not fermented to produce acids. The broth remains yellow. Methyl red is an indicator that turns red in acidic environments, that is, when glucose is fermented, and acids are produced.

Organisms indicated: *Enterobacter aerogenes*



methyl red positive – red

methyl red negative – yellow

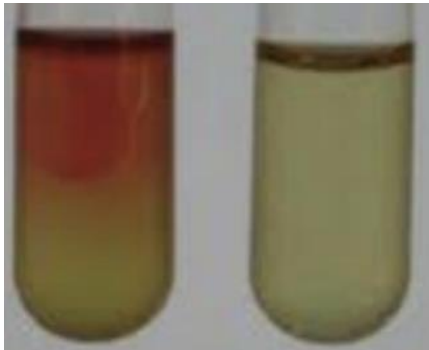
<https://www.onlinebiologynotes.com/methyl-red-mr-test-objective-principle-procedure-and-result/>

13. Voges Proskauer:

Observation: Broth is pink after the addition of Barritt's reagent

Result and explanation: Positive for alcohol fermentation. Bacteria ferment alcohol and produce acetoin. Barritt's reagent would turn acetoin pink if alcohol was fermented. For the negative result, the broth remains yellow

Organisms indicated: *Enterobacter aerogenes*, *Enterococcus faecalis*, or *Staphylococcus aureus*



positive for alcohol fermentation – pink

negative for alcohol fermentation – yellow

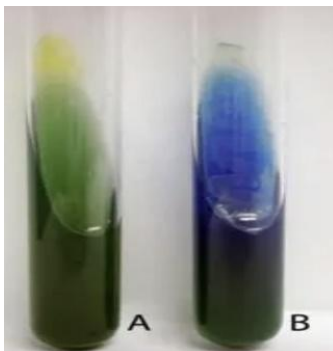
<https://microbenotes.com/author/sagararyalnepal/page/5/>

14. Citrate Utilization:

Observation: Agar is blue

Result and explanation: Positive for citrate. If bacteria metabolize citrate, then the carbon dioxide produced combines with sodium in agar, and sodium bicarbonate is produced. This causes the pH of the medium to increase. Bromomethyl blue is a pH indicator that turns from green to blue if pH increases to basic, above pH=7

Organisms indicated: *Enterobacter aerogenes*, *Salmonella typhimurium*



citrate positive – blue color

citrate negative – green color

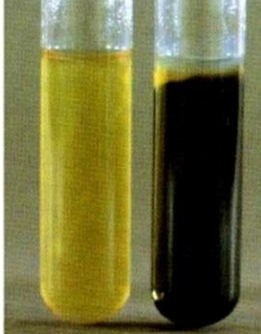
<https://microbeonline.com/citrate-utilization-test/>

15.H₂S Production:

Observation: Agar has no black color

Result and explanation: Negative for hydrogen sulfide production. No black precipitate was formed because it lacked an enzyme desulfurase. This enzyme removes sulfur from amino acids containing sulfur and releases H₂S gas. Iron in Klinger's iron agar reacts with H₂S and produces a black precipitate.

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*



H₂S positive - black precipitate

H₂S negative - no black precipitate

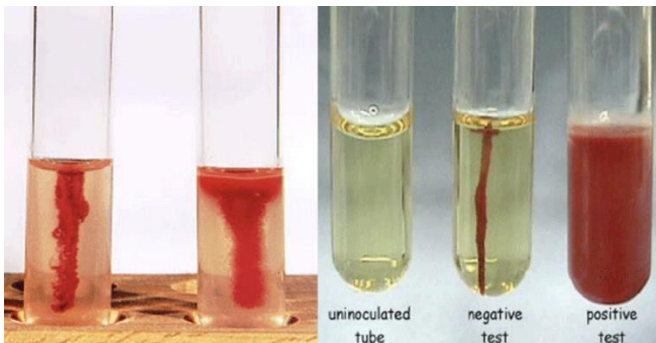
<https://microbiologyinfo.com/hydrogen-sulfide-test/>

16.Motility:

Observation: Agar is turbid

Result and explanation: Positive for motility. A deep stab is used. Each bacterium is stabbed with an inoculating needle into the tube of TSA. If the bacteria grow and diffuse from the edge, then it is a positive motility

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*



positive motility – turbid or bacteria diffuse from the edge

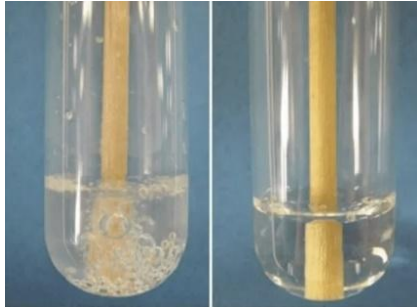
<https://microbenotes.com/motility-test-principle-procedure-and-results/>

17.Catalase:

Observation: Bubbles after hydrogen peroxide addition

Result and explanation: Catalase positive. Bubbles in the test tube. Hydrogen peroxide was converted into oxygen by an enzyme catalase

Organisms indicated: *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, or *Staphylococcus aureus*



catalase positive – bubbles

catalase negative – no bubbles

<https://microbeonline.com/catalase-test-principle-uses-procedure-results/>

Identification of Bacteria Associated with the Urinary Tract infection

The possible bacteria:

Enterococcus faecalis, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* or *Staphylococcus epidermidis*.

18. Gram reaction and shape:

Observation: Pink rods scattered around the field

Result and explanation: Gram-negative bacilli, individual. The bacteria's cell wall is made of a thin peptidoglycan layer covered by an outer plasma membrane. The decolorizing agent (alcohol) washes off crystal violet from Gram-negative bacteria, which became colorless, the counterstain, safranin, dyes the cell wall to pink color.

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*



<https://microbenotes.com/escherichia-coli-e-coli/>

19. MacConkey agar plate:

Observation: pink growth

Results and explanation: Gram-negative bacteria, positive for lactose fermentation. MacConkey agar is a selective and differential medium. Bile salts and crystal violet inhibit Gram-positive bacteria's growth and allow Gram-negative growth. Lactose allows differentiation. Bacteria that ferment lactose produce an acidic environment. Agar contains a pH indicator (neutral red). If lactose is fermented, then acid is produced, causing the neutral red to turn a pink color

Organisms indicated: *Escherichia coli*



Gram-negative bacteria, positive for lactose fermentation – pink

Non-lactose fermenting bacteria - colorless

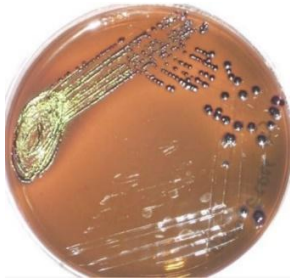
https://en.wikipedia.org/wiki/MacConkey_agar

20. Eosin-Methylene Blue agar:

Observation: metallic green growth

Results and explanation: Gram-negative bacteria, positive for lactose fermentation. Eosin and Methylene blue inhibit Gram-positive bacteria's growth and allow Gram-negative growth. They also indicate pH. Bacteria that ferment lactose produce an acidic environment. If lactose is fermented, then acid is produced, causing the bacteria to turn a metallic green color

Organisms indicated: *Escherichia coli*



Gram-negative growth - metallic green color

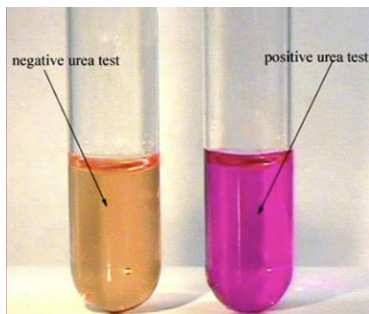
https://www.researchgate.net/figure/Escherichia-coli-colonies-on-eosin-methylene-blue-agar-Green-metallic-sheen-of-colonies_fig1_258011460

21. Urea hydrolysis:

Observation: Orange liquid in the test tube

Result and explanation: Negative urea hydrolysis. Urea broth is a differential medium to check if the bacteria produce urease that hydrolyzes urea to carbon dioxide and ammonia. Phenol red turns yellow in an acidic environment. If urea is hydrolyzed and ammonia is produced, an alkaline environment turns the media pink.

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*



<https://microbiologyinfo.com/urease-test-principle-media-procedure-and-result/>

22. Indole production:

Observation: Red ring visible after Kovac's reagent addition

Result and explanation: Indole positive. Tryptophan broth is used as a medium. If the bacteria contain an enzyme tryptophanase, then tryptophan is hydrolyzed to produce indole, pyruvic acid, and ammonia. Kovac's reagent added reacts with indole, and a red ring is formed.

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*



indole positive – red ring

indole negative – no red ring

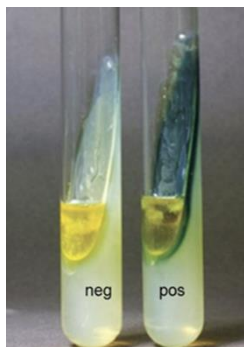
<https://microbiologyinfo.com/indole-test-principle-reagents-procedure-result-interpretation-and-limitations/>

23. Phenylalanine deamination:

Observation: No dark green color observed after FeCl_3 addition

Result and explanation: Negative for phenylpyruvic acid. Phenylalanine agar, phenylalanine deaminase medium is used to test the bacteria for deaminase production. Phenylalanine deaminase enzyme is not present to react with phenylalanine and produce phenylpyruvic acid. Addition of ferric chloride does not change the medium color to dark green.

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, or *Staphylococcus epidermidis*.



phenyl pyruvic acid positive – dark green

phenyl pyruvic acid negative – medium colorless

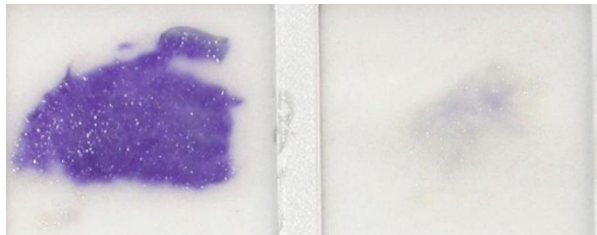
<https://www.onlinebiologynotes.com/phenylalanine-deaminase-test-principle-requirements-procedure-and-results-interpretations/>

24. Oxidase reaction:

Observation: no blue color after oxidase addition

Result and explanation: Negative oxidase activity. This test checks if the bacteria is an aerobe and possess cytochrome c and cytochrome c oxidase enzyme. Cytochrome C transports electrons to oxygen in aerobic respiration. Cytochrome c oxidase catalyzes the oxidation of cytochrome c and reduces water to form oxygen. If cytochrome C oxidase is absent, the oxidase reagent does not change color to blue.

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*, *Enterococcus faecalis*, or *Staphylococcus epidermidis*.



Oxidase positive – blue

Oxidase negative – no color change

<https://commons.wikimedia.org/w/index.php?curid=1968827>

25. Nitrate reduction:

Observation: Red color after nitrate reagents addition

Result and explanation: Positive for nitratase. Nitrate broth checks if the bacteria reduce nitrate (NO_3) to nitrite (NO_2). Nitrate reagents are added to the nitrate broth. If the medium turns red after addition, the nitrate reagents, then the enzyme nitratase reduces nitrate to nitrite, and only nitratase is present

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*



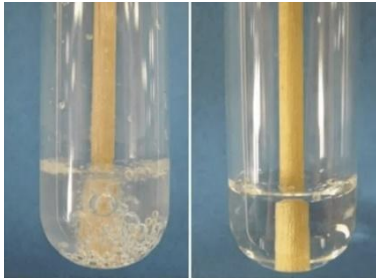
<https://microbiologyinfo.com/nitrate-reduction-test/>

26. Catalase:

Observation: Bubbles after hydrogen peroxide addition

Result and explanation: Catalase positive. Bubbles in the test tube. Added to the test tube, an enzyme catalase conversion of hydrogen peroxide into oxygen.

Organisms indicated: *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, or *Staphylococcus epidermidis*.



catalase positive – bubbles

catalase negative – no bubbles

<https://microbeonline.com/catalase-test-principle-uses-procedure-results/>