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Conceptual Paper

Laboratory Report on Advanced Diagnostic Techniques

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Abstract

On first day we have taken Orientations, how to collect samples? Why to collect? From where to collect? Which media is needed for our sample, which we selected? After all we identifies, we are taken necessary equipment's for, that is used for blood collection, like syringes, icebox, Gauze, sterilizing reagents (Alcohol) and non-coagulant test tube. Then we are going to Haramaya university sheep farm, for blood sample collection. Before we take our sample (blood from the animal/sheep), first of all we ask permission for entrance in to the farm and after the told our aim/objectives to came to these farm and after we agreed, we ask the farm worker, some crucial question that is needed for our work to be effective.

Keywords: Alcohol; Blood; Icebox; Syringes

History

- Is there is any animal that has been taken any medication recently?
- After we identify animals those are not taken any medication, we start our procedure to collect samples,
- We select animal with good body condition and we make it animals restraining
- We suspect parameters, like febrile (body temperature)
- We select the area (from animal body) to take samples:- Jugular vein and we sterilize the sites for blood collection
- We prepare our syringes and anti-coagulant test tubes and Then we injected by small syringes (that is used for human) and collect our samples, when we collect our sample we find the jugular vein slowly and then after we gate the areas, push down the blood to test tubes and we closed the test tube (sample) and put in to icebox.
- After we collect our samples, we disinfect (Alcohol) the animals and also we collect all materials that we have been used in that areas.
- Finally we bring back our sample to Haramaya university College of veterinary Medicine and we put our sample, on refrigerator.

Methods of bacteria identification

There are different ways to identifies different types of bacteria. These techniques includes:

- Isolation in pure form and cultural characteristics
- Biochemical properties
- Staining reaction.

Isolation in pure form and cultural characteristics

These can be done by clinical sample is streaked into a solid medium (like: MacConkey agar, or blood agar). In case cultural media

characteristics, these medium can be classified in to two; these are: Solid medium and liquid/fluid medium.

Solid medium

Under solid medium we can appreciate different characteristics of the bacteria's, these includes:

- **Shape:** Circular or irregular.
- **Size:** The size of the colony can be a useful characteristic for identification. The diameter of a representative colony may be measured.
- **Surface:** Smooth, rough etc.
- **Texture:** Dry, moist, mucoid, brittle, viscous.
- **Color:** Colorless, pink, black, red.

Liquid/fluid medium

- Degree of growth- Absence, moderate or abundant etc.
- Present of turbidity and its nature.
- Nature of surface growth.
- Odor.

Media preparation

First we select the sample we went to collect (Milk and fecal) samples. Then we select milk sample for mastitis detection and fecal sample for *E. coli* or *Enterobacterieae* and swab (surface swab and carcass swab from abattoirs).

So based on our samples, we prepared different Medias: - that used for sample inoculation/culture and transporting.

Culture media

- Macconkey agar
- Buffered peptone water
- Blood agar.

Macconkey agar preparation

Purpose of these media: Is used for the selective isolation, cultivation and differentiation of coliform and enteric pathogens (microbes) based on the ability to fermant lactose.

Based on general procedures, we can prepare our solution based on our interest, so we went to prepare 80ml, Here is the procedure we used.

First we calculate

If 50.03 gm are dissolved in 1000 ml, how many grams of media (Mac conkey) powder are needed for 80ml of distilled water?

50.03 gm……1000 ml

x……80 ml, by using cross cross methods.

We gate 4.02 gm, which is what we went prepare.

- We add 4.024 gm powder to 80 ml of distilled water
- Then we Bring volume to 1 liter and mix thoroughly
- We Autoclave it at 15 psi pressure at 121^0C for 15minutes.

Buffered peptone water

Used for isolation of *salmonella*, especially injured Microorganism from food sources.

Based on general procedures, we can prepare our solution based on our interest, so we went to prepare 60ml, Here is the procedure we used.

If 20 gm are dissolved in 1000 ml, how many grams of media (Buffered peptone water powder) are needed for 60 ml of distilled water?

20 gm……1000 ml

x……60 ml, by using cross cross methods

We gate 1.2 gm, which is what we went prepare.

- We add 1.2 gm powder to 60 ml of distilled water
- Then we Bring volume to 1 liter and mix thoroughly
- We Autoclave it at 15 psi pressure at 121^0C for 15minutes

Blood agar base (infusion agar)

Used for isolation and cultivation of many fastidious pathogenic Microorganism after addition of blood.

Based on general procedure we prepare our medias as follow:

If 40 gm are dissolved in 1000 ml, how many grams of media (blood agar) powder are needed for 60ml of distilled water?

40 gm……1000 ml

x……60 ml, by using cross cross methods

We gate 2.4 gm, which is what we went to prepare.

- We Suspend 40 gm in 1000 ml distilled water
- Then we heat the medium completely
- Then we Sterilize by autoclave at 15 lbs pressure for 15 minutes
- We Cool to 45 - 50^{o}C and aseptically we add 5% v/v sterile defibrinated blood
- Finally we Mix well and pour into sterile Petridish plates.

After we prepare all the above three medias we and pour into sterile Petridis plate, incubate for 24 hours.

Sample collection

First we identify the case (disease) we went to detect, and then we select Mastitis and *E. coli*. So, based on our selection, we collect Milk and fecal samples. Before we take the first of all we told to the farm physicians, why come to these farm and then we ask History of the animals, that means is there is any animals which shows symptoms of mastitis and Enterogenic (*E. coli*) disease? And then the farm physician told us yes and then we collect our samples both fecal and milk from all infected animals only. After we collect our samples, we keep aseptically and bring back to haramaya university CVM, Veterinary Microbiology lab.

Sample

- Milk sample
- Fecal samples
- Carcass and surface swab (abattoirs).

Part 1: Milk sample examination

On blood agar

On these media we inoculate milk samples. We take milk sample from two cows that are with clinical mastitis and sub clinical mastitis symptoms'. We inoculate milk sample on blood agar using the following procedures.

Procedure

- The milk was collected from cow with suspected mastitis
- The inoculating loop was sterilized by flame on Bunsen burner and cooled
- Then Small amount of milk was picked up by the loop and placed in one corner of petridish containing blood agar
- After then The loop was sterilized and a loop full sample was streaked gently across blood agar medium
- Then the loop was sterilized by flaming and cooled by stabbing at the corner of blood agar medium
- Then The loop was passed once across one end of the primary streak and streaked onto the free region of blood agar media
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak and making the third streak
- Then again then again the loop was sterilized, cooled again and streaked starting from the end of the third streak to the fourth streak and also to the large free zone on the center of the media
- Then The inoculums was labeled and incubated at 37^{o}C for 24 hours.

Interpretation

On these media both milk sample that we take from clinical and subclinical mastitis are grown. These are due to Blood agar is both differential and enriched medium. The colony displays a light to golden yellow pigment and surrounded by zones of clear beta-hemolysis. The colonies were well recognized and it is possible to perform other test for bacterial identification.

On MAC conkey agar

On these media we inoculate milk samples. We take milk sample from two cows that are with clinical mastitis and sub clinical mastitis symptoms'. We inoculate milk sample on macconkey blood agar using the following procedures.

Procedure

- The milk was collected from cow with suspected mastitis
- The inoculating loop was sterilized by flame on Bunsen burner and cooled
- Then Small amount of milk was picked up by the loop and placed in one corner of petridish of macconkey agar media
- After then The loop was sterilized and a loop full sample was streaked gently across macconkey agar medium
- Then the loop was sterilized by flaming and cooled by stabbing at the corner of macconkey agar medium

- Then The loop was passed once across one end of the primary streak and streaked onto the free region of macconkey agar media
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak and making the third streak
- Then again then again the loop was sterilized, cooled again and streaked starting from the end of the third streak to the fourth streak and also to the large free zone on the center of the media
- Then The inoculums was labeled and incubated at 37^{0}C for 24 hours.

Interpretation

There is no change of color on these media and our samples that we inoculate on these media cannot grow. This is due to MacConkey agar is a selective and differential culture media that commonly used for the isolation of enteric Gram-negative bacteria and these media contains lactose, Crystal violet and bile salts that inhibit the growth of Gram-positive bacteria. So, based on these microorganism can gram positive bacteria and can grow on other Medias.

Biochemical test

For further examination we prepare other Medias [Biochemical Test] for these milk sample examination Mannitol salt agar.

Mannitol salt agar

General principles: - If the organism is tolerant to salt it will grow. If the organism is not tolerant to salt it will not grow. If the salt tolerant organism can ferment mannitol then there will be yellow zones around the colonies. If the salt tolerant organism cannot ferment mannitol then the media will remain pink.

Preparation of mannitol salt agar

Procedure

- We suspend 5.5gram in 100ml of distilled water
- Then we heat it to dissolve completely
- After then we sterilize by autoclave at 115^{0}C for 20 minutes
- Then we cool to 45-50^{0}C
- Then we mix well and poured on plates.

After then we transfer samples that are cultured before on blood agar to mannitol salt agar and we incubate at 37^{0}C for 24hrs.

Results

On the first media there is no growth that is negative and on the second one there is growth of colonies that is positive.

Interpretation

On A, there is no change of colour as well as there no grow these means, the organism is not tolerant to salt it will not grow.

On media B there is the grow of microorganisms; these means that the organism is tolerant to salt it will grow and also there is a change in color of the media. These means the organism is salt tolerant due to these reason the organism can ferment mannitol then there will be yellow zones around the colonies.

Based on the above two images on A, there is no change of colour as well as there no grow of colony, the means, samples with clinical mastitis can be mannitol negative, these means the organism can not a staphylococcus species. So, based on the above results again we want further examination for samples with clinical mastitis (A) because of there is no grows of the colony on mannitol salt agar and due to previously grow on blood agar, we suspect this sample may be staphylococcus species, so in order to differentiate we done Edward media which is selective media for streptococci species.

Edwards's medium

Preparation

- We suspend 2.05gram in 50ml of distilled water
- Then we boil it to dissolve completely
- After then we sterilize by autoclave at 115^{0}C for 20 minutes
- Then we cool to 50^{0}C
- Then we add 5% of sterile sheep blood
- Then we mix well and poured on plates.

Results for Sample B, on Edward media - there is no growth of colonies on these Medias.

Interpretation

Edward media is selective media for streptococci species. So, organism is not streptococci species and it may be other microorganism species. Because not only staphylococcus and streptococci species can causes mastitis.

Gram staining

These method is used to differentiate either the microorganism is gram negative or gram positive. These can be performed based on the color of the colony.

- If the organism is Gram positive bacteria- violet
- If the organism is Gram negative bacteria- pink.

Gram staining procedure

- First we fix the smear
- Then we Stain with the crystal violet(primary stain) for 30 seconds
- Then we wash crystal violet off with water
- After then we add iodine for 10 seconds
- Then we wash iodine off with water
- After then we add (alcohol) decolorize with ethyl alcohol for 10-20 seconds
- After then we wash the alcohol off with water
- Then we add Counter stain/Safranin for 30 seconds
- After then again we wash the safranin off with water
- Then we dry the smear and
- Finally observe under microscopy by adding oil immersion.

Result

When we examine under microscopy we observe deep purple in color, coccus shaped and presence as clustered, which is the main characteristics of staphylococcus genus; Gram-positive bacteria.

Interpretation

Staphylococcus is a genus of bacteria that is characterized by a round shape (coccus) Gram-positive (purple), and found as in clusters that resemble a bunch of grapes. *Staphylococcus* Circular, pinhead colonies which are convex with entire margins. This gram positive coccus often produces colonies which have a golden-brown color.

Enzymatic test

Coagulase test

For the detection of *Staphylococcus* species:

- First we prepare clean, glass microscopic slides.
- Then aseptically we place a drop of hydrogen peroxide onto slide.
- After then by using a sterile inoculating needle we transfer a visible amount of organism from blood agar media to the hydrogen peroxide on slide.
- Then we mix.

Results

Finally we observe production of bubbling or bubble formation, which indicates a positive catalase test.

Interpretation

The presence of vigorous oxygen bubbling indicates that the hydrogen peroxide has been broken down by the enzyme catalase. The organism is staphylococcus species. All Staphylococci produce strong catalase activity.

Part 2: Fecal sample examinations

We collect fecal samples from one cow and one calf. Both of them are diarrheic. We take history either the animal is treated or not, because in order to gate the colony. The ways of preparation of media; both MacConkey agar and blood agar is similar to the ways that we prepare for milk samples.

Culturing of fecal sample on blood agar

On these media we inoculate fecal samples. We take fecal sample from one cow and one calf, which are both of them, has diarrhea.

Procedure

- The fecal sample was collected from cow and calf with diarrhea
- The inoculating loop was sterilized by flame on Bunsen burner and cooled
- Then Small amount of fecal was picked up by the loop and placed in one corner of petridish containing blood agar
- After then The loop was sterilized and a loop full sample was streaked gently across blood agar medium
- Then the loop was sterilized by flaming and cooled by stabbing at the corner of blood agar medium
- Then The loop was passed once across one end of the primary streak and streaked onto the free region of blood agar media
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak

- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak and making the third streak
- Then again then again the loop was sterilized, cooled again and streaked starting from the end of the third streak to the fourth streak and also to the large free zone on the center of the media
- Then The inoculums was labeled and incubated at 37 ^{0}C for 24 hours.

Results

We observe colonies with big circular, gray and moist.

Interpretation

Based on the above results, the colonies of this organism is *E. coli*. The Colonies are appeared with morphological characteristics big, circular, gray and moist colonies and Beta (β) hemolytic colonies are formed.

Culturing of fecal sample on mac conkey agar

MacConkey agar is both a selective and differential medium frequently used in culture testing. It contains crystal violet dye and bile salts, both of which inhibit the growth of most gram-positive bacteria. It contains lactose (a sugar) and neutral red indicator (a pH indicator which is yellow in a neutral solution, but turns pink to red in an acidic environment), which allow for differentiation.

On these media we inoculate fecal samples. We take fecal sample from one cow and one calf, which are both of them, has diarrhea.

Procedure

- The fecal sample was collected from cow and calf with diarrhea
- The inoculating loop was sterilized by flame on Bunsen burner and cooled
- Then Small amount of fecal sample was picked up by the loop and placed in one corner of petridish of macconkey agar media
- After then The loop was sterilized and a loop full sample was streaked gently across macconkey agar medium
- Then the loop was sterilized by flaming and cooled by stabbing at the corner of macconkey agar medium
- Then The loop was passed once across one end of the primary streak and streaked onto the free region of macconkey agar media
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak
- Then again The loop was sterilized, cooled again and streaked starting from the end of the secondary streak and making the third streak
- Then again then again the loop was sterilized, cooled again and streaked starting from the end of the third streak to the fourth streak and also to the large free zone on the center of the media
- Then The inoculums was labeled and incubated at 37 ^{0}C for 24 hours.

Results

We observe colonies with pink color. In general on MacConkey agar, Escherichia coli would ferment the lactose producing acid and would form colonies pink to red in color.

Interpretation

Samples are grown on Macconkey agar with Colonies that have circular, moist, smooth and of entire margin and appear flat and pink. They are lactose fermenting colonies. Growth on the plate indicates the organism, Escherichia coli, is not inhibited by bile salts and crystal violet and is a gram-negative bacterium. The pink color of the bacterial growth indicates E. coli is able to ferment lactose.

Eosin methylene blue agar (EMB)

EMB Agar (Eosin Methylene Blue Agar) is recommended for the isolation and differentiation of gram negative enteric bacteria from clinical and nonclinical specimens. It is useful in differentiating gram positive and gram-negative bacteria. It helps in the isolation and differentiation of enteric bacilli and gram-negative bacilli. It is used in testing the quality of water, especially in determining if the water is contaminated by harmful microorganisms. It differentiates microorganisms in the colon-typhoid-dysentery group. EMB media assists in visual distinction *Escherichia coli*, other nonpathogenic lactose-fermenting enteric gram-negative rods, and the *Salmonella* and *Shigella* genera. It also helps in the isolation and differentiation of lactose fermenting and non-lactose fermenting enteric bacilli.

Media preparation

General procedure said that, Dissolve 37.5 gram of powder of nutrient to 1000 ml of distilled water, this is general procedure, but we done by 50ml. so based on general procedure and our interest, the calculation of these media is as follow:

If 37.5 gram is dissolved in ----------1000 ml

How many gram of media powder is needed----------for 50 ml?

When we calculate, we gate 1.875 gram, so based on our desire, the procedure we follow to prepare these media is as follow:

- We suspend 1.875 gram in 50 ml of distilled water
- Then we heat with frequent agitation
- Then we cool to 50^{o}C
- Then we mix well and poured in to sterile plates.

After all we inoculate our cultured colonies, which are grown on blood agar to EMB media, for isolation and differentiation of the microorganism.

Results

We observe colonies with Blue-black and growing with green metallic sheen, which is *Escherichia coli*.

Interpretation

Rapid lactose fermentation produces acids, which lower the PH. This encourages dye absorption by the colonies, which are now colored purple-black, which is the colony of *E. coli*. In general on EMB *E. coli* are grown with distinctive metallic green sheen, this is due to the metachromatic properties of the dyes, *E. coli* movement using flagella, and strong acid end-products of fermentation).

Part 3: Carcass swab and surface swab

Peptone water sugar media

Used for This medium is recommended as a diluent for the homogenization of food samples containing suspected contaminants such as *Salmonella.*

Preparation

- We Add 1.5mg powder to 200ml of distilled water
- Then mix thoroughly
- Then we heat and bring to boiling
- Then we Autoclave at 15psi pressure at 121^{o}C for 15 minutes
- Finally we distribute in to sterile test tube.

After all procedure we done, we culture our sample, which is surface swab and carcass swab, for 24hr.

Results

We observe visible turbidity.

Rappa Port Vassiliadis Media: these media is transporting media.

Preparation

- We Suspend 2.5 grams of the medium in 100ml of distilled water.
- Then we Heat with frequent agitation until complete dissolution.
- The we Dispense on test tube and
- Finally we sterilize at 115ºC for 15 minutes.

XLD (xylose lysine deoxycholate)

Used for: Selective media for isolation and differentiation.

Preparation

- We Suspend 5.6grams of the medium in 100 ml of distilled water.
- Then we Heat with frequent agitation.
- Then we Transfer immediately into a water bath at about 50ºC.
- Then we cool immediately on water bath at 45-50^{o}C
- Then we pour into sterile Petri plates.

Then we inoculate surface swab and carcass swab that we have been taken haramaya university slaughter house, and we incubate for 24 hours.

Results

Surface swab, colony are grown with red with black center, which is *Salmonella*.

Meat swab, colonies are red colonies with black center.

Interpretation

Based on the above results that we observe, our sample is well grown on the XLD media whith colonies characterized by red colonies with black center. These media contains Lysine that is used to differentiate the *Salmonella* group from the non-pathogens. *Salmonella* rapidly ferment xylose and exhaust the supply. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red coloration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation.

Nutrient agar

Used for: For the cultivation of non-fastidious microorganisms.

Procedure

General procedure said that, Dissolve 28 gram of powder of nutrient to 1000 ml of distilled water, this is general procedure, but we done by 150 ml. so based on general procedure and our interest, the calculation of these media is as follow:

If 28 gram is dissolved in ----------1000 ml

How many gram of media powder is needed----------for 150 ml?

We gate 4.2 gram

- Then by cross crossing we gate gram, this means we dissolve 4.2 gram of nutrient agar in to 150 ml distilled water.
- Then we dissolves well and heating
- Then we cool in water bath.

Then we dispense on Petridis and we take grown colony from Macconkey agar and also carcass swab and surface swab colonies from XLD and then we put in thirty seven degree centigrade for 24 hours.

Interpretation

The first test tube or A (surface swab) is *salmonella* species, because the colony of the organism grows with the following characteristics:

- Shape-------circular
- Surface---------smooth
- Color----------greyish white
- Structure-------translucent opaque.

Biochemical test for swab (colony) sample

Triple sugar iron

Procedure

- We use inoculation needle and we sterilize it and cool it
- Then we sterilize or heat the tip of test tube on the flame
- Then we pick the suspected organism from the tip of test tube by inoculation needles
- Then finally we done slant or zigzag on the culture media.

Results

Interpretation

The production of acid in the slant of the tube during glucose fermentation oxidizes rapidly, causing the medium to remain orange red or revert to an alkaline PH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube since it is under lower oxygen tension.

- Glucose fermentation only.
- H_2S: *Salmonella*
- Glucose, sucrose, and/or lactose fermenter with gas: *E. coli.*

MR-VP medium

Used: Recommended for the performance of methylene red and voges proskaur tests used in differentiation of coli-aerogenes group. To test the ability of the organism to produce and maintain stable acid end products from glucose fermentation and to overcome the buffering capacity of the system this is a qualitative test for acid production.

Preparation

- We Suspend 3.4 grams of the medium in 200 ml of distilled water.
- Then Mix well.
- Then we heat slightly to dissolve completely.
- After then we Distribute it in tubes in 10 ml
- Finally we sterilize at 121ºC for 15 minutes.

We culture carcass swab and surface swab on these media, and there is no grown of colonies in these Medias.

Results

- MR positive - Red colour
- MR negative - yellow colour

Simon citrate medium

Used for: Simmons Citrate Agar is used to differentiate enteric Gram-negative bacilli on the basis of sodium citrate utilization as a source of carbon and inorganic ammonium salt utilization as a source of nitrogen.

Preparation

- We suspend 2.4 gram in 100 ml of distilled water
- Then we heat
- We dispense on test tubes
- We sterile on autoclave at 121°C for 15 min and
- We set it as slopes because to detects the ability of an organism to utilize citrate as the sole source of carbon and energy for growth and ammonium salt as the sole source of nitrogen.

Result

The test organism is cultured in a medium which contains sodium citrate, an ammonium salt and the indicator bromothymol blue. Growth in the medium is shown by turbidity and a change in color of the indicator from light green to blue, due to alkaline reaction following citrate utilization.

Interpretation

Positive test shows development or Growth of deep blue color on the slant of the medium indicates positive test.

Indole test

Procedure

- We Inoculate Tryptone broth with the test organism and incubate for 24 hrs. at 37^0C
- The we add a drops of Kovac's reagent down the inner wall of the tube.

Results

- **Interpretation:** Based on our results, the presence or occurrence of bright red color with ring formation at the (top of the test tube) or interface of the reagent and the broth within seconds after adding the reagent is show that there is the presence of Indole and is called Indole positive test. Based on this description and picture below; so based on our results.
- **Indole Positive:** *E. coli*
- **Indole Negative:** *Salmonella* spp.

Gram staining

Is used to determine whether a strain(s) is present in a sample, it's necessary to stain the sample. Here, Gram stain is used as it helps distinguish between the gram positive and gram negative bacteria in a sample.

- Place the slide on a staining rack and flood the smear with crystal violet for about one minute
- Slightly tilt the slide and rinse with water (distilled or tap water) gently
- Flood the sample with iodine and allow the slide to stand for about 1 minute
- Slightly tilt the slide again and rinse using tap or distilled water (gently).
- Tilt the slide and decolorize using 95% ethyl alcohol/acetone - decolorizing should be done by applying the alcohol in drops for about 8 seconds
- Rinse with water gently
- Blot the slide dry
- Place the slide on microscope and view the sample.

Results

When seen under the microscope, we observe colonies that will appear pink in color.

Interpretation

The main purpose of Gram stain is to classifying bacteria based on the color. This staining technique distinguishes between two main types of bacteria (gram positive and gram negative) by imparting color on the cells. So, based on the result that we observe under microscope, the microorganism is *E. coli,* Which is a Gram-negative bacterium. This is because they stain negative using the Gram stain. Gram-negative bacteria, *E. coli* have an additional outer membrane that is composed of phospholipids and lipopolysaccha-

rides. The presence lipopolysaccharides on the outer membrane of bacteria give it an overall negative charge to the cell wall. Because of these properties, E. coli does not retain crystal violet during the Gram staining process.

Antimicrobial susceptibility tests

We have done antimicrobial sensitivity test for *E. coli* and *Salmonellosis*.

- First we prepare Mueller Hinton agar
- Preparation of Mueller-Hinton Agar
- Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
- Then we autoclaving it and allow it to cool in a 45^{o}C on water bath.
- Then we it Pour on the petridish.
- Then we stored at room temperature
- Then the antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface
- Then plates are inverted and placed in an incubator for 24 hrs.

Interpretation

Antimicrobial susceptibility testing results are interpreted using the laboratory standard methods recommended by the guidelines. The most common guidelines are Committee of Laboratory Standard Institute (CLSI) and European Committee of Antimicrobial Susceptibility Testing guidelines (EUCAST).

Based on the above results and general interpretation that can be formulated by CLSI we can gave the following descriptions.

- **Resistant:** When a micro-organism is defined as clinically resistant by a level of antimicrobial susceptibility which results in a higher than expected likelihood of therapeutic failure.
- **Susceptible:** When a micro-organism is defined as clinically susceptible by a level of antimicrobial susceptibility which results in an improved, or the desired, therapeutic outcome.
- **Intermediate:** When a micro-organism is defined as clinically intermediate by a level of antimicrobial susceptibility which results in an indeterminate therapeutic outcome.

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