

# Examination of Milk (Bacterial)

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4<sup>th</sup> Class



# Etiology of Mastitis

Mastitis may caused by:

Contagious pathogens

\**Staphylococcus aureus*  
\**Streptococcus agalactiae*

Opportunistic pathogens

Coagulase negative  
staphylococci:  
\**Staphylococcus hyicus*  
\**Staphylococcus epidermidis*

Environmental pathogens

\**Escherichia coli*  
\**klebsiella*  
\**Streptococcus uberis*  
\**Streptococcus dysagalactiae*

# Sample collection

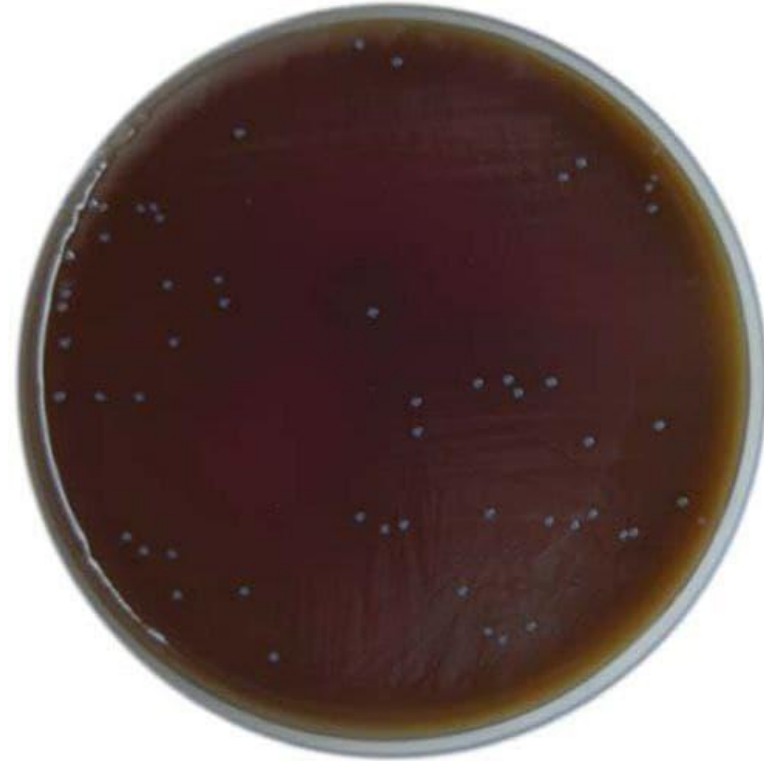
- Aseptic milk sampling:
  1. Wear clean gloves.
  2. Use towel soaked with warm water and soap to clean the udder and teats.
  3. Dry the udder and teat after cleaning.
  4. Use teat dip (disinfectant contain 0.2% iodine) for 30 second , then dry with clean towel.
  5. Wipe the teat orifice with 70% alcohol.
  6. Strip the teat horizontally in to sterile test tube.
  7. Label the sample with animal data (identification number, age, date ...etc.).
  8. Transfer the samples immediately to the laboratory using ice box.

# Culture Media used to Isolate of Bacteria from Milk

- Edward's medium
- Blood agar
- Mannitol salt agar
- Glycine-Tellurite Agar
- Hotis Test

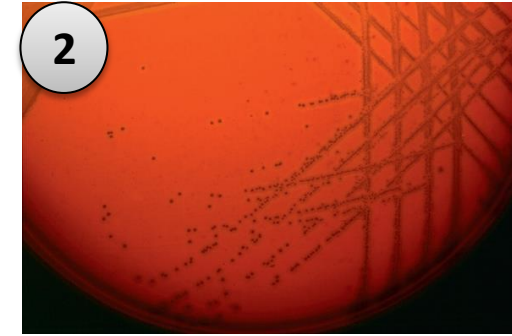
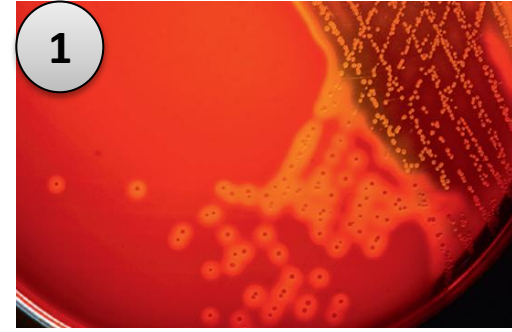
# Edward's medium

- Contain Aesculin, blood and Crystal violet
- Inhibits *Staphylococci*
- Coliform bacteria are readily distinguished by their characteristic black colonies.
- *Streptococci* produce characteristic colonies as follows:
  1. *Streptococcus agalactiae*, gray-blue colonies
  2. *Streptococcus dysgalactiae*, gray or grayish-blue colonies
  3. *Streptococcus uberis*, brown colonies.



# Blood Agar

- Contain 5-10% Sheep Blood
- Used to identify the type of hemolysis produced by hemolytic bacteria
- Types of hemolysis are:
  1. ( $\beta$ ) Beta-hemolysis: a clear zone of hemolysis around the colony
  2. ( $\alpha$ ) Alpha-hemolysis: a zone of greening or of partial hemolysis
  3. ( $\gamma$ ) Gamma-hemolysis: no hemolysis



# Mannitol Salt Agar

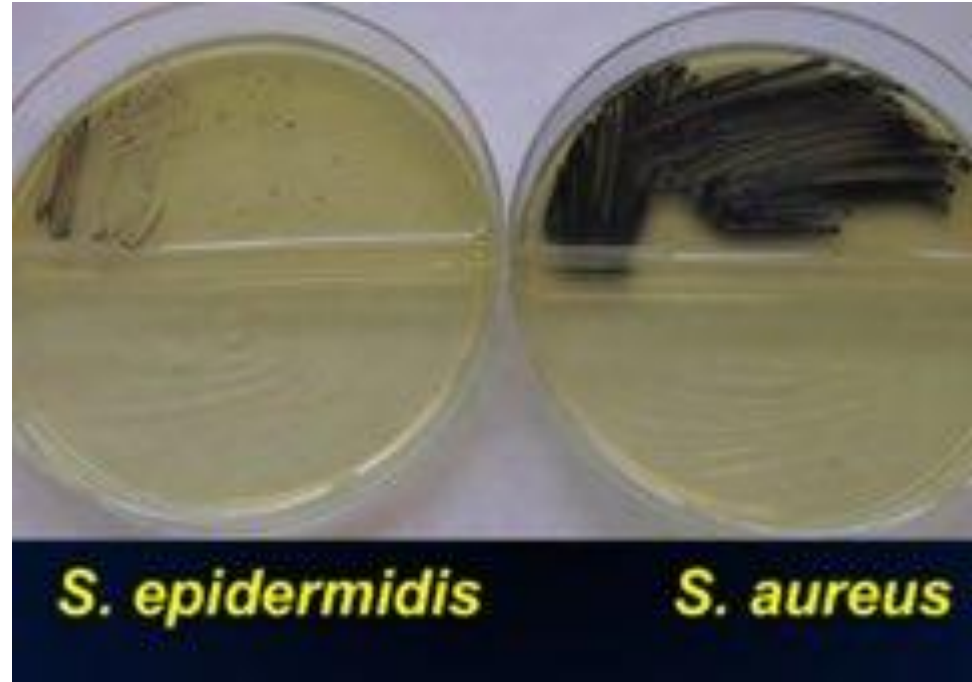
- Contain 7.5-10% Sodium Chloride.
- Selective medium for the isolation of *Staphylococci*.
- Differentiate between *Staphylococcus aureus* that ferment mannitol and other non fermentating *Staphylococci*.



*Staphylococcus aureus* (left)  
and *Staphylococcus epidermidis*  
(right)

# Glycine-Tellurite Agar

- Selective medium for *Staphylococci*.
- *Staphylococci* appear as black colonies, whereas other organisms produce a clear to colorless colony.





# Hotis Test

- This test helps to detect presence of *Streptococcus agalactiae*.

**9.5ml (milk) + 0.5ml (Bromocresol purple solution)**

**Incubate  
for 24  
hours at  
37 °C**

## **Results:**

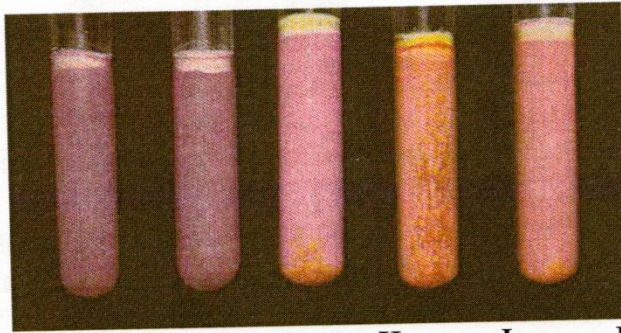
- **Light purple, no change: Normal milk.**
- **Yellow colonies on the slide of the test tube or yellow sediment: *Streptococcus agalactia*.**
- **Red or rusty flakes (agglutinated colonies) on the slide, or red sediment: Presence of *Staphylococci* or *Micrococi* (72 hours incubation).**

\* **Note:** If more than one type of organisms is present, or when the sample is contaminated, as combination of changes may obscure test reaction



A B C D E

- *A, negative tests: color remains unchanged, no flake formation*
- *B, C, D, and E, characteristic of the several changes typical for *Streptococcus agalactiae*, acid formation and few to many yellow or brown flakes*

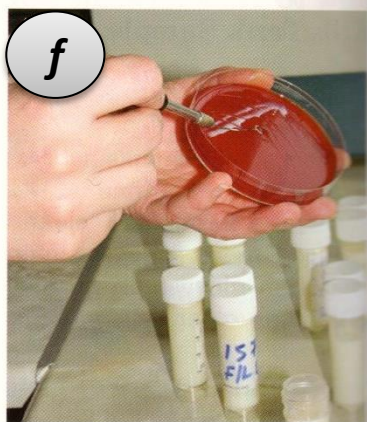
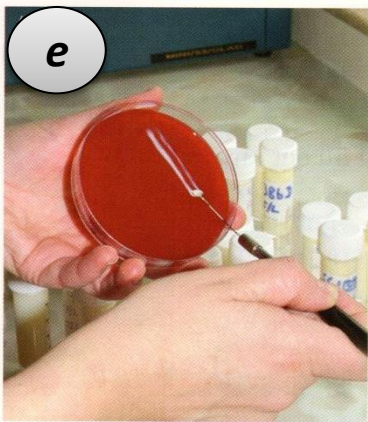
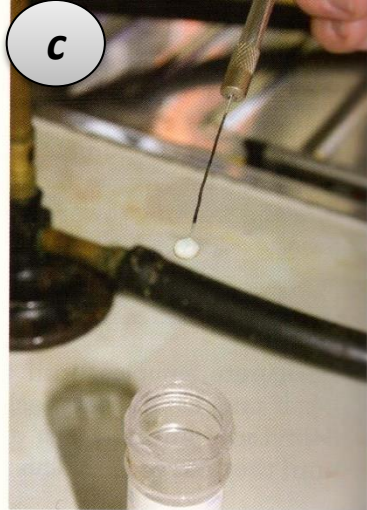


F G H I J

- *F, negative tests: color remains unchanged, no flake formation*
- *G, purple column, white flakes (diphtheroides)*
- *H, I, slightly acid, rust-colored flakes (*Staphylococcus aureus*)*
- *J, slightly acid, yellow sediment, no flakes (nonhemolytic staphylococci and streptococci other than *S. agalactiae*)*



**Hotis Test**



*Plating up. (a) Flame loop to sterilize. (b) Platinum loop holds 3 $\mu$ ml of milk. (c) Pick up aliquot of milk. (d) Plate on to media. (e) Spread primary inoculum. (f) Flame loop and streak out to dilute to form single colonies.*

- Incubate at 37 °C for 24 hours
- Gram stain
- Biochemical test (Catalase, Coagulase, Oxidase, antibiotic sensitivity test)