

# Indicator Organisms

# Indicator organisms

- ▶ Group of microorganism use to reflect the quality and safety of a process food product
  - ▶ In water
    - ▶ Vibrio cholera
    - ▶ Salmonella typhi
    - ▶ Shigella spp
    - ▶ Hepatitis
  - ▶ In food
    - ▶ Coliform

# Criteria for use of an organism as an indicator

- ▶ Must be associated with feces
- ▶ Should not be a natural contaminant
- ▶ Easy to detect
- ▶ Test should be less complex than test for pathogen
- ▶ Growth limit under conditions
- ▶ Should not survive processing
- ▶ Processing should have same effect on pathogens

# Suggested indicator organisms

- ▶ Fecal organism
  - ▶ Fecal coliform
  - ▶ Escherichia coli
- ▶ Enteric bacteria
  - ▶ Shigella spp.
  - ▶ Salmonella spp.

# Coliforms

- ▶ Coliform indicates processing contamination
- ▶ Coliform can grow on food processing equipment or in environment
- ▶ Several genera are considered to be coliforms
  - ▶ Escherichia
  - ▶ Enterobacter
  - ▶ Klebsiella
  - ▶ Citrobacter
- ▶ Source of coliform
  - ▶ Fecal group --GI tract of humans and animals
  - ▶ Non-fecal group --Soil and plant

# Coliforms

- ▶ Coliform group
  - ▶ Gram negative, rod shape, aerobic or facultative aerobic
  - ▶ non endospore former
  - ▶ ferments lactose at 30-37 °C for 24-48 hrs
- ▶ Fecal coliform
  - ▶ found only in GI tract
  - ▶ can grow at elevated test temperature
  - ▶ ferment LST at 44.5 °C for 24 hrs

# Coliforms

- ▶ Methods for isolation of coliforms

- ▶ Presumptive test

- ▶ Liquid media (MPN)

- ▶ LST (Lauryl Sulfate Tryptose)

- ▶ Solid media

- ▶ VRBA (Violet red bile agar)

- ▶ Confirm test

- ▶ Liquid media (MPN)

- ▶ BGLB (Brillant Green Lactose Bile broth)

- ▶ EC (EC broth)

# Presumptive test of coliforms

## ▶ LST

- ▶ triplicate tubes for each dilution
- ▶ incubate at 32-35 °C for 24-48 hrs
- ▶ coliform grow---turbidity and gas

## ▶ VRBA

- ▶ surface plate method---spread on plate
- ▶ incubate at 32-35 °C for 24-48 hrs
- ▶ red, purple colonies with ring of precipitate bile salt around colonies

# Confirmation of coliforms

- ▶ BGLB

- ▶ Incubate at 32-35 °C for 24-48 hrs
- ▶ Coliform report as MPN

- ▶ EC

- ▶ Incubate at 44.5 °C for 24 hrs
- ▶ Fecal coliform report as MPN

# Identification of coliforms

- ▶ Select colony or positive tubes and streak onto PCA slants
- ▶ Identify the microorganism
  - ▶ Gram stain
  - ▶ Biochemical test
    - ▶ IMViC test
      - ▶ Indole test
      - ▶ Methyl red
      - ▶ Voges-Proskauer
      - ▶ Citrate utilization
  - ▶ Selective media
    - ▶ EMB agar (Eosin Methylene Blue Agar)

# Identification of coliforms

IMViC test: use to differentiate between the coliform group

- ▶ Indole test
  - ▶ Bacteria degrade tryptophan to indole
- ▶ Methyl red
  - ▶ Bacteria produce acid from glucose
- ▶ Voges-Proskauer
  - ▶ Detect acetylmethylcarbinol from glucose
- ▶ Citrate utilization
  - ▶ Bacteria utilize citrate as a carbon source

# Identification of coliforms

## IMViC test

Organism	Indole	Methyl red	Voges-Proskauer	Citrate
<i>E. coli</i>	+	+	-	-
<i>E. aerogenes</i>	-	-	+	+

# Identification of coliforms

## ▶ Selective media

### ▶ EMB (Eosin Methylene Blue) medium

- ▶ Selective and differential plating for isolation and identification of Gram-negative enteric bacteria
- ▶ Eosin and methylene blue used as indicators
  - ▶ Bacteria ferment lactose
- ▶ Sucrose used to detect coliform that ferment sucrose
  - ▶ E. coli → dark colony with black center and green metallic sheen
  - ▶ Enterobacter → pink colony
  - ▶ Klebsiella → dark center colony

# Coliforms

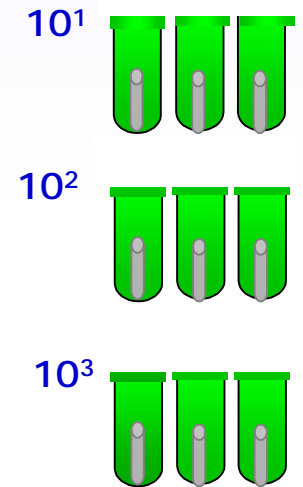
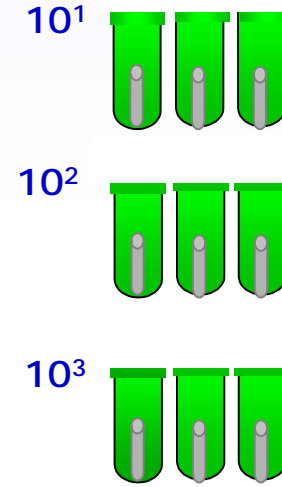
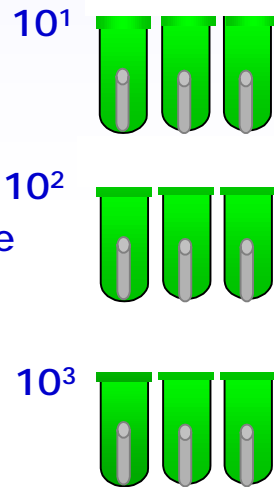
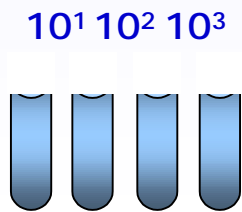
## Presumptive test

TST medium

## Confirm test

BGLB medium

EC medium

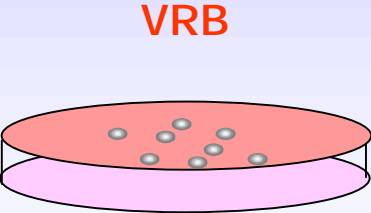


**Positive:** Gas production  
Change of medium color

Coliform  
MPN/ml

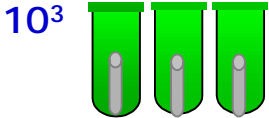
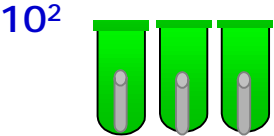
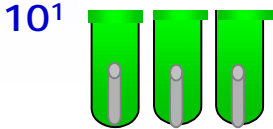
Fecal coliform  
MPN/ml

# Coliform Count



OR

BGLB/EC



Innoculate

PCA slant

