



**Agriculture  
and Markets**

# Dairy Waters: Testing and Follow up

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# Dairy Waters

- Why is water important?
- Types of water in the dairy industry
  - Testing requirements and proper reporting
- Approved test procedures
- Follow up on an unsatisfactory sample
- Role of laboratory



# Importance of Water in the Dairy Industry

- Water is used for cleaning equipment and utensils on farms and in processing plants
  - Must be safe and sanitary to prevent contamination
- Animal consumption on dairy farms
- Sanitizing and sterilization of equipment
- Water is necessary for hand washing
- Used for heating and cooling products on farm and during processing; culinary steam
- Used to push or convey product during processing; rinsing of product (such as cottage cheese curd)

# Individual Water Supplies

- Individual Water Supplies
  - Farms, plants, receiving and transfer stations, milk tank truck cleaning facility single service plants
- Tested for: total coliform; *E. coli* on same sample if positive for total coliform
- Testing frequency:
  - Initially;
  - After any repair, modification or disinfection of supply;
  - Every 3 years thereafter –farm;
  - Every 6 months - plant, receiving/transfer station, milk truck cleaning facility,
  - Every 12 months – single-service containers and/or closures plants
- If results TNTC or CG, sample is invalid and shall have a Heterotrophic Plate Count

# Reclaimed Water: Category I Water – Potable Water Purposes

- Category I water used as potable water
  - Reclaimed from milk and milk products; heat exchangers or compressors
  - Used in plants
- Tested for: total coliform; *E. coli* on same sample if positive for total coliform
- Testing frequency:
  - Initially;
  - After any repair, modification or disinfection of supply;
  - Every 6 months
- If results TNTC or CG, sample is invalid and shall have a Heterotrophic Plate Count

# Category II Reclaimed Water and Recirculated Cooling Water

- Category II Reclaimed Water
  - Limited purposes – not potable
  - Farms, plants, receiving/transfer stations, single service manufacturers
- Recirculated Cooling Water
  - Used in plate or tubular coolers and/or heat exchangers
  - Sweet water, chill water, glycol
- Tested for: total coliform; *E. coli* confirmation not required
- Testing frequency :
  - Initially;
  - After any repair, modification or disinfection of supply;
  - Every 6 months
- If results TNTC or CG, sample is invalid and shall have a Heterotrophic Plate Count

# Pasteurized Equivalent Water

- Water that comes in contact with pasteurized milk and/or milk products
  - Samples collected daily for two weeks following approval of initial installation
  - Sampled at least once every six month period thereafter
  - Daily testing shall be conducted for one week following repairs or alterations to system
- *E. coli* testing is required on samples positive for total coliform

# Why test for coliforms?

- What are coliforms?
  - Facultative anaerobic, gram-negative, non-spore forming, rod-shaped bacteria; ferment lactose with gas and acid production and develop colonies with distinctive characteristics on Endo-type agar medium
  - Present in the environment and in feces of all warm-blooded animals and humans
- Coliform bacteria are used as indicators of water quality
  - Presence in drinking water indicate pathogens could be in the water system
- Coliform testing results, together with results from engineering or sanitary surveys, provide best assessment of sanitary quality of source water



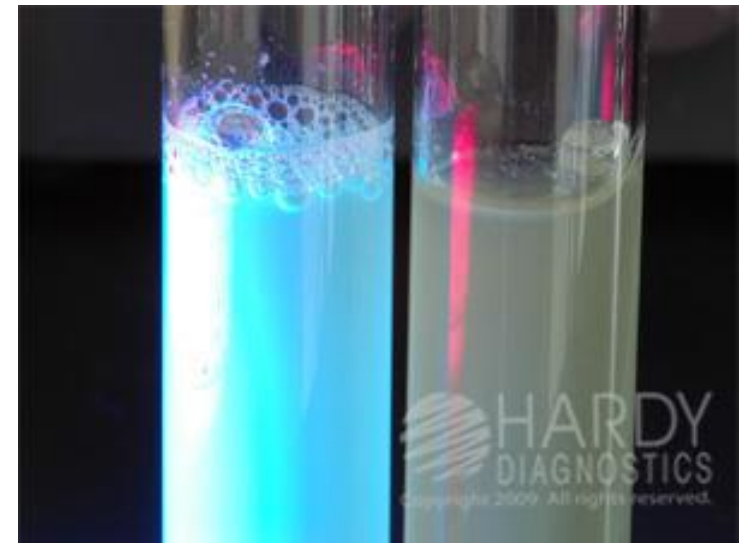


# Approved Test Methods

- M-a-98 – April 2020
- Multiple Tube Fermentation
  - Most Probable Number and Presence/Absence
- Membrane Filtration
- Chromogenic Substrate
  - Colilert
  - Colilert-18
  - Colisure
  - Modified Colitag
  - E\*Colite
- Heterotrophic Plate Count

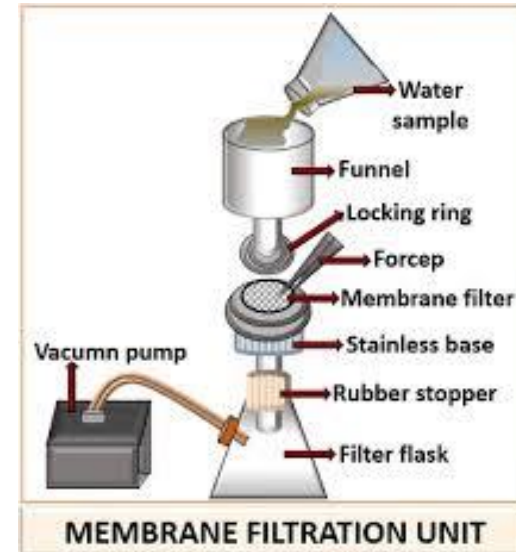
# Multiple Tube Fermentation

- Presence/Absence or Most Probable Number
- Can be used on all types of water
  - Individual water/source water, Category 1 Reclaimed Water, Pasteurized Equivalent Water – require *E. coli* confirmation with EC MUG and UV lamp
- Gas production or turbidity during presumptive test (DS-LST) must be confirmed using BGLB (all tests) and EC-MUG if *E.coli* confirmation is required
  - Turbidity with no gas in BGLB during confirmation – sample is invalid and a re-sample is needed for HPC.
- Results and Interpretation:
  - Multiple tubes:  $<1.1/200\text{mL}$  – not found;  $\geq 1.1/100\text{mL}$  - positive
  - Presence/Absence:  $<1/100\text{mL}$  – not found;  $\geq 1/100\text{mL}$  – positive
  - Specify results as total coliform (and *E. coli* if testing is required)



# Membrane Filtration

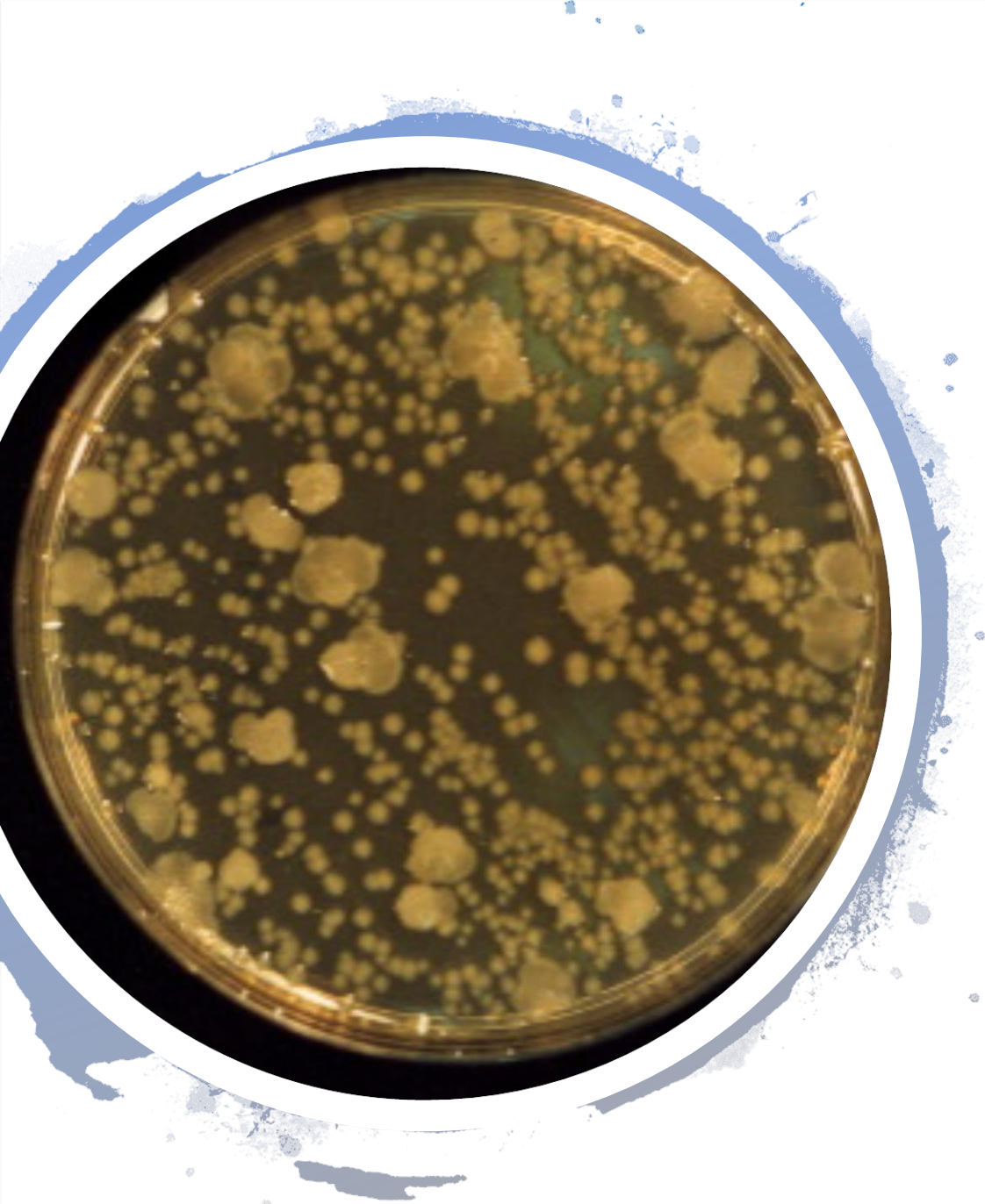
- Can be used for all waters
  - *E.coli* confirmation requires use of EC MUG and UV lamp
- Has limitations when testing waters with high turbidity or large numbers of background bacteria
- Develop colonies with distinctive characteristics on M-Endo media
  - Red colonies with metallic (golden green) sheen – typical
  - Dark red, mucoid, or nucleated colonies without metallic sheen – atypical
  - Generally, pink (non-mucoid), blue, white or colorless colonies lacking sheen are non-coliforms
- Confluent growth or TNTC: total number of bacterial colonies is >200 per membrane; colonies not distinct enough to count accurately
  - Invalid
- Growth must be confirmed using LST and BGLB (all); EC MUG for samples requiring *E. coli* confirmation
- Interpretation:
  - <1/100mL is Not Found
  - ≥1/100mL is positive; fluorescence is positive for *E. coli*



# Chromogenic Substrate (MMO-MUG and XGAL - MUG)

- Most Probable Number and Presence/Absence
- Approved for source/individual supply water systems, Category 1 reclaimed water (plant), Pasteurized Equivalent Water
- Uses hydrolysable chromogenic and fluorogenic substrates to detect enzymes produced by total coliforms and *E.coli*
  - Chromogen – coliform bacteria present
  - Fluorogen – *E. coli* present
- Colilert, Colilert-18, Colisure, Modified Colitag
  - Use comparator; yellow is positive for total coliform; red or magenta for Colisure
- E-Colite
  - Blue-green is positive for total coliform
- Fluorescence under UV light is positive for *E. coli*
- Reporting: <1/100mL is not found; ≥1/100mL is positive – specify total coliform and *E. coli*





# Heterotrophic Plate Count

- Can be used with all types of water
- Estimate number of live, culturable heterotrophic bacteria in water
- Results reported in CFU/mL (colony forming units)
  - Colonies may arise from pairs, chains, clusters, or single cells
- Similar to Standard Plate Count
  - Incubate at  $35\pm 0.5^{\circ}\text{C}$  for  $48\pm 3$
- Interpretation:
  - Not Found -  $< 500$  CFU/mL
  - Positive -  $\geq 500$  CFU/mL

# Interpreting Results:

- Not found for total coliform = satisfactory system
- Positive for total coliform only = at risk system
- Positive for total coliform and *E. coli* =unsatisfactory system
- TNTC or CG = invalid sample; follow up with Heterotrophic Plate Count
  - HPC <500 cfu/mL = satisfactory; NF
  - HPC ≥500 cfu/mL = unsatisfactory; positive

# Following Up on “At Risk” Water System

- What is an “at risk” system?
  - Positive for total coliform, not *E. coli*
  - HPC >500 cfu/mL on previously invalidated sample
- If system is deemed at risk:
  - Physical Inspection
  - Corrections
  - Bacteriologically satisfactory sample
- Inspection must be completed and documented within 30 days of the positive sample result. No inspection = Unsatisfactory water system
- If water still tests positive for total coliform, regulatory agency will conduct an inspection

# Following Up on Unsatisfactory Water System

- What is an unsatisfactory water system?
  - Positive for total coliform **and** *E. coli*
  - Water system inspection done completed
- If water is considered unsatisfactory:
  - Physical inspection – Regulatory Agency
  - Corrections made
  - Bacteriologically satisfactory sample
- Follow up on unsatisfactory reclaimed/recirculated system:
  - Same as unsatisfactory potable water system



# Laboratory's Role

- Receiving sample
  - Samples transported and maintained at 0-4.5°C
  - Unrefrigerated samples rejected if transit exceeds 6 hours
  - Transit time ≤30 hours
  - Need 100mL for testing
    - Underfilled sample containers rejected; Overfilled sample containers without sufficient air space for mixing rejected
- Testing completed within 30 hours of collection or within 2 hours of receipt
- Approved test method used
  - Chromogenic substrate – source waters only
  - Tinted water sample – color may interfere with Colilert or Colisure testing
- Results reported at each step of incubation; reported and interpreted correctly
  - 1.1/100mL vs 1/100mL
  - Positive or NF for total coliform and Positive or NF for *E. coli* (when required)
  - **Do not** test recirculated or Category II water for *E.coli*
  - **Do not** report *E.coli* results as NF if testing is not performed. Only individual water supply/source water, Reclaimed Cat. I water and PEW samples positive for total coliform must be tested for *E. coli*
- *E.coli* confirmation completed when required
- Prompt notification when results are unsatisfactory

# Questions?

- Pasteurized Milk Ordinance
  - 2019 revision has been released
- Dairy Waters FDA/NCIMS form 2400m
  - Current revision 10/19
- M-a-98
- Standard Methods for the Examination of Water and Wastewater
  
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