

# BACTERIOLOGY OF WATER, MILK AND AIR

---

**Dr Rituparna Saha**

*Dept of Microbiology, UCMS & GTHB*

# Bacteriology of Water

- Drinking water has to be visually acceptable, clear, colourless, without disagreeable taste or odour
- Should be safe
- Waterborne major diseases are...
- Reach through fecal or sewage pollution

**Table 1. Examples of waterborne pathogens**

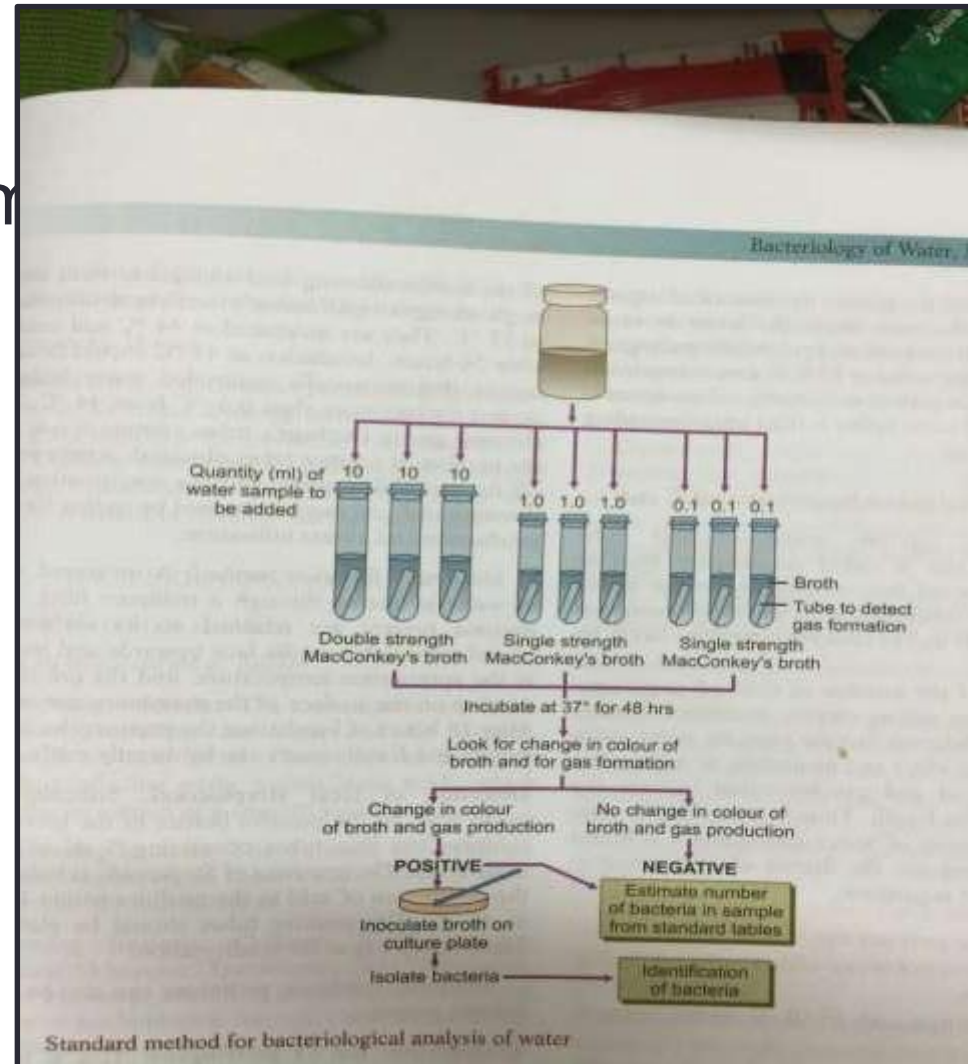
<b>Viruses</b>	<b>Health effect</b>
adenovirus	conjunctivitis, diarrhea, encephalitis, respiratory and heart disease
astrovirus	diarrhea
norovirus	diarrhea, 'stomach flu'
coronavirus	diarrhea
hepatitis A virus	hepatitis
rotavirus	diarrhea
enterovirus	paralysis, meningitis, rash, fever, myocarditis, respiratory disease, diarrhea
reovirus	respiratory disease
<b>Bacteria</b>	<b>Health effect</b>
<i>Aeromonas hydrophila</i>	sepsis, gastrointestinal illness
<i>Yersinia enterocolitica</i>	gastroenteritis
<i>Salmonella (non)/typhi</i>	paratyphoid fever, gastroenteritis, typhoid fever
<i>E. coli</i> O157:H7	gastroenteritis, vomiting, hemolytic uremic syndrome, hemorrhagic colitis
<i>Shigella</i> spp.	dysentery
<i>Campylobacter</i> sp.	gastroenteritis, nervous system disorders
<i>Helicobacter pylori</i>	ulcers
<i>Legionella pneumophila</i>	Legionnaires Disease, Pontiac fever, pneumonia
<i>Vibrio cholerae</i>	diarrhea
<b>Protozoa</b>	<b>Health effect</b>
<i>Cryptosporidium parvum</i>	cryptosporidiosis
Microspora	gastroenteritis
<i>Giardia lamblia</i>	giardiasis
<i>Entamoeba histolytica</i>	dysentery
<i>Cyclospora cayetanensis</i>	gastroenteritis
<i>Acanthamoeba</i>	eye infections
<i>Toxoplasma gondii</i>	similar to mononucleosis
<i>Naegleria fowleri</i>	amoebic meningoencephalitis

# Tests done are

- Plate count:
  - Counting no. of colonies in pour plate cultures
  - On NA plates at 37 degree C for 1-2 days
  - And at 22 degree C for 3 days
  - 37 : most likely human origin; index of dangerous pollution
  - 22: mainly saprophytic; indication of decomposing organic matter in water

# Detection of Coliform and E.coli

- Presumptive coliform tube technique



- Differential coliform test:
  - Eijkman test to determine if coliforms are E.coli
  - After presumptive test, S/C to single strength Mac medium warmed to 37 deg C
  - Incubate at 44deg C in waterbath
  - Gas in Durhams tube within 24 hrs: E.coli
  - Further confirmation by indole at 44 deg C

- Membrane filtration method:
  - Measured qty of water filtered thru millipore filter
  - Bacteria will be retained on surface
  - Filter placed on media face upwards and incubated
  - Colonies that develop are counted
  - Presumptive coliform and E.coli count after 18 hrs incubation

# Bacteriological examination of water

- Should be regular, periodical procedure
- Drinking water should be free from any pathogenic microorganisms
- Primary test employed is: Coliform detection
- Classification of drinking water:

Class	Presumptive coliform count/100ml	E.Coli count / 100ml
Class 1 Excellent	0	0
Class 2 Satisfactory	1-3	0
Class 3 Suspicious	4-10	0
Class 4 Unsatisfactory	>10	0.1 or more



- **Detection of fecal streptococci**
  - Subcultures from positive bottles in tubes containing 5 ml glucose azide broth
  - Str.fecalis: production of acid within 48 hrs at 45deg C
  - Further confirmation: BE
- **Millipore membrane**
- **Enzymatic methods**
  - For coliforms
    - $\beta$ -galactosidase: specific for coliform bacilli
    - $\beta$ -glucoronidase: E. coli specific

- Examination of **Cl.perfringens**:
  - Water heated followed by multiple tube cultures (Differential Reinforced Clostridial Medium)
- Test for pathogenic bacteria: Pseudomonas
- Viruses in water
- Protozoa in water

# Bacteriology of Milk

## Types of bacteria in milk

Acid forming bacteria	Str lactis and Str fecalis, lactobacilli
Alkali forming bacteria	Alkaligenes spp; Achromobacter
Gas forming bacteria	Coliforms commonest. Cl perf, Cl butyricum
Proteolytic bacteria	Spore bearing aerobes eg. B.subtilis, B.cereus, P.vulgaris, staph etc
Inert bacteria	No visible change in milk.
Human milk	Breast milk contains small nos. of staph.epid, staph.aureus, etc.

- **Milkborne diseases**

- Mostly TB, *Brucellosis*, *Coxiella*, *Streptococcus* Infections and Staph food poisoning, Salmonellosis and Q fever.
- Diphtheria, Cow pox, HFMD, *Leptopirosis*, *Campylocabter*, Anthrax

# METHODS FOR DISINFECTION/STERILIZATION OF MILK

1. **THERMIZED MILK:** 57-68 deg C for 15 sec (**Methylene blue test**)
2. **PASTEURIZATION:** HOLDER METHOD (63 deg C for 30 min)/ FLASH (72 deg C for 20 sec)  
hence does NOT effectively kill: Bacterial spores, *Coxiella*, *Mycobacterium*, some toxins
3. **UHT:** 135 deg C for 1 sec (**Viable count test:  $\leq 1000$ /mL after incubating on yeast extract medium for 48 hrs**)
4. **STERILIZATION:** 100 deg for extended period to pass **TURBIDITY TEST**

# Bacteriological examination of milk

## **VIABLE COUNT:**

- Serial Plate dilution in YE milk agar
- Incub at 30 or 21 deg c for 72 hrs
- Number of colonies multiplied by dilution factor give COLONY COUNT

## **COLIFORM COUNT:**

- Serial dilution of milk inoculated in 3 tt MacConkey broth with Durham's tube
- Incub. at 37 deg C for 48 hrs and acid+gas looked for

# Bacteriological examination of milk

<b>Viable count</b>	<b>By doing plate counts with serial dilutions of milk sample</b>
Test for coliform bacilli	Same as done for water
Methylene blue reduction test	Simple substitute for viable count. Reduction of methylene blue by bacteria in milk. Rate of reduction = degree of bact contamination Raw milk safe if no reduction in 30 mins
Resazurin test	Dye resazurin, blue to pink color change Done for 10 mins
Phosphatase test	Phosphatase enzyme deactivated if proper pasteurisation of milk done
Turbidity test	If sterilised properly, all heat coagulable proteins precipitated. If ammonium SO <sub>4</sub> then added, filtered, boiled for 5 mins, there is no turbidity. Distinguish between pasteurisation and sterilisation

# Examination of specific pathogen

## • Tubercle bacillus

- Milk 3000rpm x 30 mins.
- Sediment --- LJ medium/2 guinea pigs.
- Observe animals for 3 months.
- Even culture can also be done

## • Brucella

- Inoculate cream on serum dextrose agar / centrifuged deposit i.m. in guinea pigs
- Animals:
  - ANTIBODIES: Milk ring test/ whey agglutination test
  - sacrificed after 6 weeks and tested for antibodies. Spleen inoculated in culture media

## • Examination of Cl.perfringens:

- Incubate different qty of milk in litmus milk medium (anaerobically) at 37deg C x 5days and look for stormy fermentation



# Bacteriology of Air

## Measurement of air contamination

- Sedimentation method

- Open plates of SBA exposed to air for 1 hour and then incubated for 37 deg C for 24 hrs

- Slit sampler

- Known volume of air directed onto plate through a slit 0.25 mm wide; plate mechanically rotated

- Bacteriological examination of Environmental dust
- Sweep plate method
  - For personal clothing, linen etc.
  - Culture plate rubbed over fabric surface
- Dust sampling
  - Moist cotton swabs collect dust from floor, wall, furniture
  - Inoculated in RCM; S/C on plates
  - For Clostridium tetani

Thank you!!