MICROBIOLOGICAL EXAMINATION METHODS OF FOOD AND WATER

An Industrial Training Report submitted
for the partial fulfillment of the Degree of Master of Science

By

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[M.Sc. (Microbiology), Semester IV]

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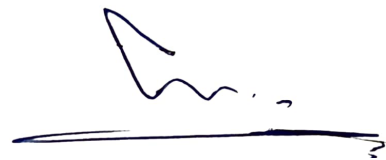

2020-21

ANNEXURE 1

(On letterhead of the Industry)

C E R T I F I C A T E

This is to certify that this training report entitled "**Microbiological Examination of Food and Water**" was successfully carried out by Miss. Dhruvi Vekariya towards the partial fulfillment of requirements for the degree of Master of Science in Microbiology of Atmiya University Rajkot. It is an authentic record of her own work, carried out by her under the guidance of Mr. Divyesh Marviya for a period of 3 months during the academic year of 2020-21. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

Name & Signature of the
Head of the Department

Name & Signature of the
supervisor

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ANNEXURE 2

D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled **"Microbiological Examination of Food and Water"** is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Dhruvi Vekariya

Date   (Name and signature of Student)

A K N O W L E D G E M E N T

As always said ," Success is not the job of one person, but it requirs support of all."

*I am greatly indebted to our respected **Pujya Tyagvallabh Swamiji**, Honorable Secretary, Sarvodaya Kelavani Samaj, Rajkot and our Principal **Dr. K. D. Ladva** who has taken keen interest in our academic progress & also provided the required facilities for the successful completion of the project work .*

*I express my deep gratitude towards **Dr. Shivani Patel**, Head of Department of Microbiology for her continuous support.*

*I also express my deep gratitude towards my internal guide **Dr. Mousumi Das.***

*I wish to express my sincere thanks to **Mr. Divyesh Marviya (Microbiologist, Envitro Laboratories Pvt. Ltd.)** for her guidance and useful suggestion, which helped me in completing my work . I am very much thankful to **Ms. Hardika Ukani (Microbiologist, Envitro Laboratories Pvt. Ltd.)** for constant support in facilitating the information and Requirement thought my journey.*

*I am also grateful to **Mr. Sunil Sangani (Director, Envitro Laboratories Pvt. Ltd.)** for helping me every time in any work .*

I would like to acknowledge the support of my colleagues our group members and all my classmates for helping me and cooperating with me.

Lastly but not the least, I wish to thank my family for raising me to believe that I can accomplish anything I put my mind to, and providing the support and encouragement that I needed to make that true .

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A B B R E V I A T I O N

DCA	deoxycholate citrate agar
EMB	eosin methylene blue
VRBL	violet red bile glucose or lactose
PCA	plate count agar
CYGA	chloramphenicol yeast glucose agar
NA	Neutrient agar
PBS	phosphate buffer solution

IS	Indian standard
TSI	triple sugar iron
BSA	bismuth sulphite agar
RV	Rappaport-Vassiliadis soya peptone broth
BGB	Brilliant green bile broth
LSB	Lauryl tryptose broth
EVAD	Ethyl violet Azide Dextrose Agar

A B S T R A C T

Microbiological Examination Methods of Food and Water is provides an overview of standard microbiological culture methods for the examination of food and water, adhered to by renowned international organizations.

Foods are subject to contaminant by wide variety of microorganisms which include bacteria, fungi, yeasts. In the present study we have emphasized the examination of various foods ,beverages and water was done and noted. Microorganisms associated with the spoilage were identified by microbiological examination of each type of foods. Such microorganisms were also isolated in pure culture and were used for future studies,

INTRODUCTION

1.0 History

Envitro Laboratories Pvt. ltd. is a full analytical & testing & research services provider company serving for food, beverage, soil, agricultural products, building and construction materials testing and environmental pollution monitoring and control for various industries, where all the in house technical work is performed and conducted. The business was started by entrepreneurial food technologist and microbiologist, Sc. Sunil Sangani, who has had a distinguished and successful executive career as a food scientist and product development director in the food, beverage, sports nutrition, pollution control and dietary supplement industry. As a biology and food science major, Mrs. Rutu Sangani also efforting best for microbiological analysis of food products. Envitro laboratories Pvt. Ltd. own a set of laboratories which are is accredited for ISO 17025 with ISO 9001 and DMI (AGMARK). The Laboratories approved CAB with the National Accreditation board for testing and calibration laboratories of india and Accredited by NABL, APEDA, DMI, AGMARK, GPCB, FDA, GERI, AND ISO.

2.0 Laboratory Test Services

1. Food testing

- Milk and milk products
- Cereal and cereal products
- Spices
- Oils and fats
- Fruits and vegetables product

- Beverages
- Ready to cooked food
  2. Environmental testing
- Agricultural products
- Water testing
- Drinking water
- Dialysis fluid or water
- Borewell water
- Waste water (ETP)
  3. Cosmetics testing
  4. Pharmaceutical testing
  5. Swab sampling analysis
  6. shelf life assessment

3.0 departments of laboratory

1. microbiological testing department
2. physical testing department
3. chemical testing department

microbiological testing department

a. changing room area
b. media preparation area
c. autoclave area
d. inoculation area
e. incubation area
f. observation area
g. discard area

a. changing room area

where wear the apron, mask, gloves, specs, and sanitize before and after leaving the room.

b. Media preparation area

  i. Passbox
  ii. Weigh balance

      iii.   Media

      iv.   Oven

      v.   Glasswares or apparatus

      vi.   Refrigerator

      vii.   pH meter

c. autoclave area

    media and glasswares are sterilized or autoclave.

d. Inoculation area

      i.   Laminar air flow

      ii.   Biosafety cabinet

      iii.   Air handling unit

e. Incubation area

      i.   BOD incubator

      ii.   Environmental shaker

      iii.   Hot air oven

      iv.   Waterbath

      v.   Deep freeze storage

f. Observation area

      i.   Microscope

      ii.   Colony counter

      iii.   UV cabinet

g. Discard area

    Where all media are discard and washing are done.

Introduction

  food and water is an integral part of our daily lives, and without food and water life itself would cease to exist. Water is essential for all forms of life and certain human activities. In the course of survival, certain living organisms may contaminate water required for use by others. Foods that are consumed daily, has a minimum shelf life and are more prone to be contaminated by microorganisms resulting in food spoilage which ultimately lead to diseases of various kind. All food should be safe and free from contamination and spoilage at all points in its journey from its source until it reaches the consumers. food-borne illness is a rising cause of morbidity in all countries and the list of potential food-borne microbial pathogens keeps increasing. In India an estimated 4,00,000 children below five years age die each year due to diarrhea.

Objectives
Why control water Quality?

    Health and survival of man and other organisms depends on the purity of the water they use. Different measures are used to access and control water quality with varying degrees of relevance and acceptability.

What is Microbiological quality of food ?

    Bacterial count in prepared food and water is a key factor in assessing the quality and safety of food. Refers to the levels of occurrence of microorganisms in the final product. Indicates the amount of microbial contaminants it has, a high level of contamination indicates low quality of food and its handling more likely
to transmit diseases. If the microbiological testing result is within the required limits, then the water / food is said to be microbiological quality for human consumption (APHA et al. 2012). It also reveals the level of hygiene adopted by food handlers in the course of preparation of such foods.

Materials and methods
1. Water analysis (drinking water, dialysis fluid or water, borewell water)

- Indicator Organisms
- Total Coliforms:
- Gram -ve, rod shaped, include bacteria that are found in the soil, in water that has been influenced by surface water, and in human or animal waste. Not necessarily fecal bacteria.
- *Fecal streptococci*:
- Gram +ve, aerobic cocci usually in short chains. They are the group of the total coliforms that are considered to be present specifically in the gut and feces of warm-blooded animals.
- *Escherichia coli* (*E. coli*):
- Is the major species in the fecal coliform group. *E. coli* is generally not found growing and reproducing in the environment.

1. Microbiological test
    I. Detection of *E.coli* and coliforms bacteria by membrane filteration method (EMB and VRBL) and Fecal streptococci in waste water (SBA) (IS 5404:1984 and IS 5887:1999)

1.Food analysis

Why microbiological analysis of foods?
One of the most important reasons for analyzing foods from both the consumers and the manufacturers standpoint is to ensure that they are safe. A food may be considered to be unsafe because it contains harmful microorganisms (e.g., Listeria, Salmonella). Foods contaminated with pathogenic microorganisms do not look bad ,taste bad or smell bad. Disease that result from contaminated food are of two types.
· Food borne intoxication
· Food borne infection

a. Microbiological test
   Quantitative analysis
    i.Enumeration of Yeast and Mold count (TPC- viable count and CYGA- Yeast and Mold) (IS 5404:1984 and IS 540

Qualitative analysis
      i.Detection of *salmonella spp.*
      ii.Detection of *shigella spp.*
      iii.Detection of *S. aureus*
      iv.Detection of *Listeria monocytogenes*
2.Swab sampling

Material
Pipettes
Tips
Membrane filter
Flask
Burner
Filter assembly
Wireloop
Forcep
Methanol
Slides
Stains

Equipments
Weigh balance
pH meter
autoclave
laminar air flow or biosafety cabinet
incubator
microscope
colony counter
UV cabinet

Media and reagents
EMB
VRBL
PCA
CYGA
SBA
OXFORD
XLD
DCA
MACCONKEY
SELENITE F BROTH
BGB
TSI

TRYPTONE WATER
BUFFER PEPTONE WATER
UREA AGAR BASE
PHOSPHATE BUFFER

Methods

I.Detection of *E. coli* and coliforms bacteria by membrane filtration method (EMB and VRBL) and *Fecal streptococci* in waste water (SBA) (IS 5404:1984 and IS 5887:1999)

Procedure:

a) Filter the about 100 ml water through membrane filter chamber (pore size – 0.45µ).
b) Place the membrane filter paper on appropriate nutrient medium (EMB & VRBL media for water, SBA media for waste water).
c) Incubate it for 24 to 48 hrs.
d) Observe the colonies on plate.
e) E. coli shows green metallic colonies on EMB plate, coliforms shows reddish purple colonies on VRBL media and fecal streptococci shows dark red colonies on SBA media shows positive results.
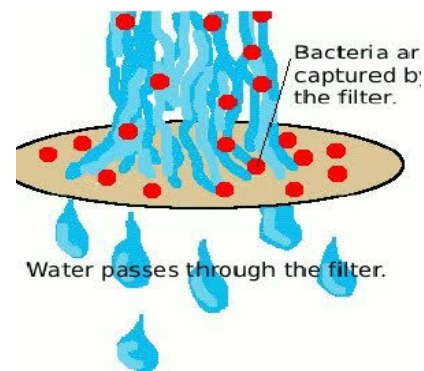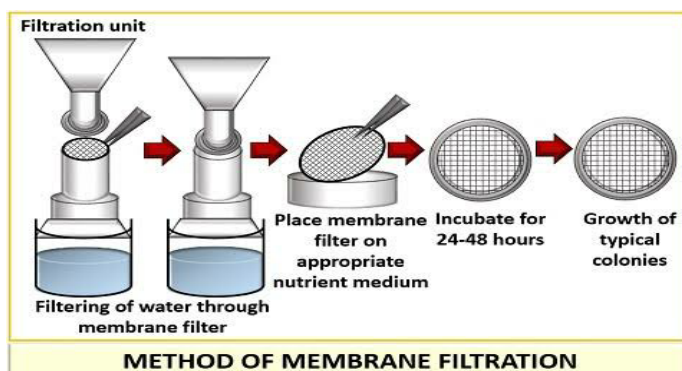
1.Food analysis

A.Microbiological test

Quantitative analysis

I.Enumeration of Yeast and Mold count (TPC- viable count and CYGA- Yeast and Mold) (IS 5404:1984 and IS 5402: 2012)

Total Viable Count

This method is applicable to the enumeration of viable aerobic bacteria (psychrophilic, mesophilic and/or thermophilic
bacteria) in foods. Figure shows the growth of bacteria on Plate Count Agar. The significance of this bacteria, however, varies markedly according to the type of food product and the processing it has received.

Procedure

Mix 10g sample into 90ml autoclaved distilled water and mix well.
Prepare 10 fold serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}.
Inoculate 0.1 ml of each dilution into PCA and spread over it.
Incubate at 30°C for 24 to 72 hour.
Colonies are produced showing positive results.

Yeast and Mold Count

This method is applicable to the enumeration of viable yeasts and molds in foods and food Ingredients, It may also be used to confirm the viability of apparent yeast and mold material Scraped from food plant equipment and the manufacturing environment. Both yeasts and molds cause various degrees of deterioration and decomposition of foods. Molds, such as *Aspergillus, Rhizopus,* and *Penicillium*, are responsible for the spoilage of cured meats. Some molds, in the right conditions, produce *mycotoxins. Aspergillus flavus (A. flavus)* molds of importance potential foodborne pathogens. They are involved in different forms of diseases, including allergies to fungal antigens, production of toxins, or direct invasion of hosts.
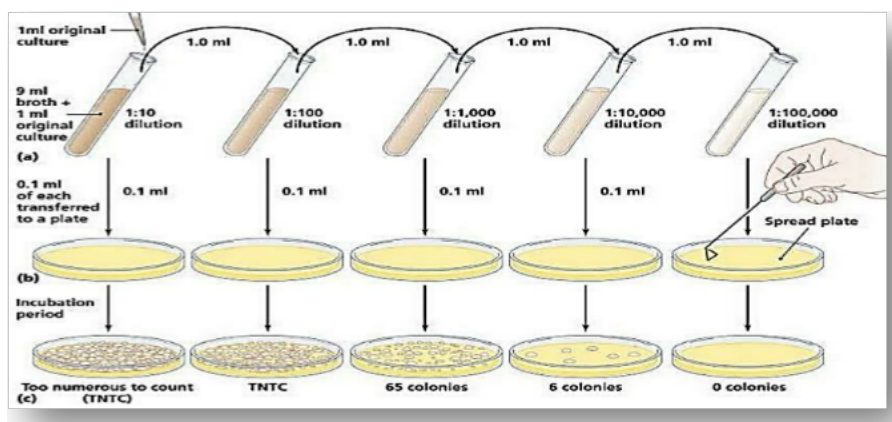
Procedure

Take 10 ml or g sample and inoculate into 90 ml autoclaved distilled water.
Take 1 ml from it and add into 9 ml distilled water and make serial dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}.
Take 0.1 ml from each and spread on to CYGA.
Incubate the plates for 24, 48 and 72 hrs. at 25°C temperature.
Observe the colonies on CYGA plates and count the colonies.

Qualitative analysis
I. Detection of *salmonella spp.*

Salmonella spp.

Salmonella is a rod-shaped, generally motile, non-spore forming, Gram-negative bacterium. Salmonella are considered among the most important enteric foodborne pathogens whose presence in the food constitutes

a severe health hazard. potentially present in most raw meats. In food processing, Salmonella infection is of concern in raw poultry swine, and ready to eat products. Salmonella are heat and acid-sensitive. Salmonella not only survives drying, but also becomes more heat-resistant with drying and is more of an issue in non-fermented dried meats, such as jerky, and whole meat cuts. Salmonella remains viable for a long time in frozen foods. Many outbreaks of human illness have been associated with the consumption of raw or inadequately heat treated milk or their dairy products.

Who can get Salmonella?
You can get Salmonella from eating a variety of foods. Salmonella can be found in a variety of foods including chicken, vegetables, eggs, fruits, sprouts, beef, pork – and even processed foods, such as frozen pot pies and stuffed chicken entrees. Contaminated foods usually look and smell normal, which is why it is important to detect this organism from food. Salmonellosis is a type of food poisoning caused by the Salmonella enterica bacterium.

Procedure
Take 25 g or ml sample and mix in 225 ml buffer peptone water.
Incubate it at 37°C for 16 to 20 hour.
If growth observed than transfer 1 ml culture into 100 ml RV medium.
Incubate it at 37°C for 16 to 20 hour.
Next day streak on XLD and BSA incubate at 37°C for 24 hours.
Pink colonies (lactose non fermenting) with black centered (H_2S production) colonies on XLD and Black colonies on BSA media showing positive results.

Biochemical test
Make slant of TSI, urea and broth of L-lysine, VP and indole.
Inoculate the colonies into it and check the result after 16 to 20 hour.
TSI lactose, sucrose, indole, urea and VP are showing the negative result and TSI glucose (acid+gas), H_2S production and L-lysine showing positive result.

II.Detection of *shigella dysenteriae.*

Shigella dysenteriae.
all members of Shigella are aerobic and facultative anaerobes. Grow readily in culture media at pH 6.4 to 7.8 at 10 °C - 40 °C, with optimum of 37 °C. After 24 hours incubation, Shigella colonies reaches a diameter of about 2 mm. The colonies are circular, convex, colorless, but moderately translucent with smooth surface, and entire edges.

Procedure
Inoculate the 25 g sample in 225 ml tetrathionate broth and incubate at 37°C for 24 hour.
Next day loopfull culture spread or streak on DCA plates and incubate at 37°C for 24 hour.
Opaque, even margins colonies found on DCA , than transfer into MacConkey agar and incubate at 37°C for 24 hour.
Next day observe the colonies and transfer on N-Agar media and incubate for 24 hour.

Biochemical tests

Make slant of TSI, urea and N agar (for catalase) and broth of Indole.

Inoculate the colonies and check the result after a day.

TSI H_2S production, urea, TSI lactose are showing negative and catalase as well as indole showing positive results.

III.Detection of *S. aureus*

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacterium. It is a non-motile, non-spore forming facultative anaerobe that can grow with or without oxygen. staphylococcal food poisoning is caused by the consumption of a heat-stable enterotoxin produced as a byproduct during the growth of certain strains of *S. aureus.* It becomes a problem when competitive microbes are removed by cooking or inhibited by high-salt levels. associated with mucous membranes (nose and throat) and is commonly found on the skin and hair of healthy humans and animals. MID - Greater than 100,000 cells per gram (less than 1.0 microgram of enterotoxin A). It should not be detected in 25gm of any types of food. If detected, considered as potentially hazardous and may result in food borne illness if consumed.

Procedure

Take 10 g sample and 90 ml 0.1% peptone and mix properly.

Than 10 ml of aliquot was transfer into cooked salt medium and streak on BPA media.

Incubate both medium at 37°C for 24 to 30 hour.

From the salt medium make subcultures on BPA and incubate for a day at 37°C.

Observe greyish black colonies on BPA media.

IV.Detection of *Listeria monocytogenes*

Listeria monocytogenes

 rod-shaped, non-spore-forming, Gram-positive bacterium. Listeriosis occurs among the elderly, pregnant women (outcome-spontaneous abortion, premature delivery). It is motile and can grow in cool (temperature range of 0-45 °C). diabetics, people on kidney dialysis, and the immunocompromised (bone marrow transplant patients, cancer patients, etc. can also suffer from listeriosis. large proportion of uncooked meat, milk, egg, seafood's and fish, as well as leafy vegetables contain *Listeria monocytogenes* (Ray, 2004).

Source ready-to-eat (RTE) hot dogs, deli meats, pate and meat spreads, fermented raw meat sausages, and raw poultry and meat .

procedure

25 g sample added in 225 ml half Fraser broth and mixed it well.

Incubated at 30°C for 24 hour.

Streaked on Oxford and PALCAM agar and incubated at 30°C for 48 hour.

1.1 ml incubated HFB was transferred in to 10 ml secondary Fraser broth. And incubated at 37°C for 48 hour.

Streaked on Oxford agar.

Observed greenish sheen with black hole and sunken center colonies on Oxford agar.

3.Swab sampling

Procedure
Take swab sampling tube filled with 1ml peptone water in it.
Make 10cm x 10cm square on the surface from which we have to collect.
Spread swab vertically and horizontally.
Put the swab in peptone water containing tube and perform TPC method.
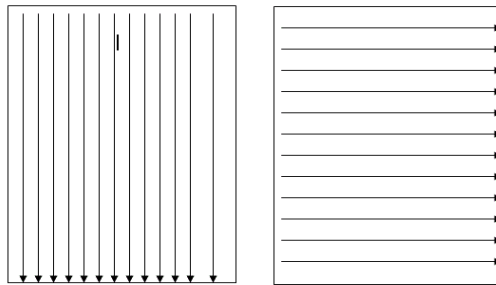
RESULT
Analysis of water and its interpretation

No.	Type of water	Test	Interpretation
1	Drinking water	*E. coli and* Coliforms	Present / absent
2	Bore well water	*E. coli and* Coliforms	Present / absent
3	Waste water	*Fecal streptococci*	Present / absent
4	Dialysis fluid	TPC/CYGA(as per FSSAI range)	…….CFU/….ml
5	Tap water	*Salmonella spp.*	Present / absent
		Shigella dysenteria	Present / absent
		S. aureus	Present / absent
		Listeria monocytogens	Present / absent

Analysis of food and its interpretation

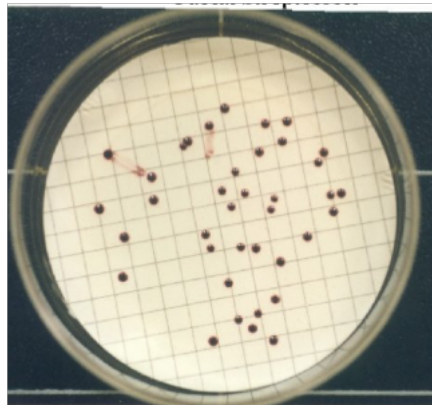| No.	Type of food	Test	Interpretation
1	Any food	TPC/CYGA (as per FSSAI range)	.......CFU/ ....gm
		Salmonella spp.	Present / absent
		Shigella dysenteria	Present / absent
		S. aureus	Present / absent
		Listeria monocytogenes	Present / absent

Membrane filtration method (*E. coli*, coliform, *Fecal streptococci*)
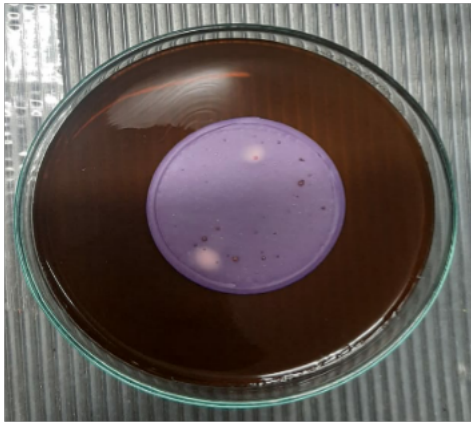
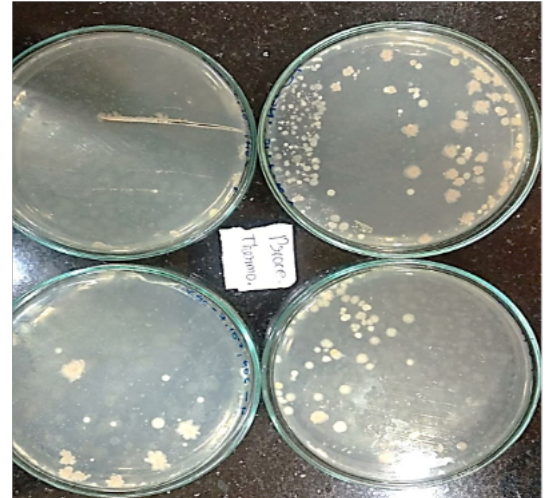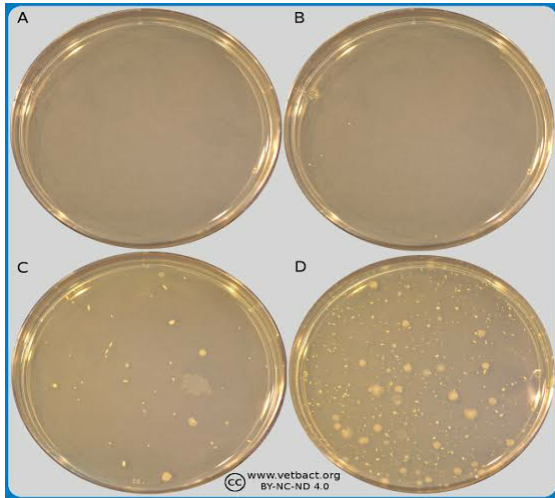E. coli on EMB
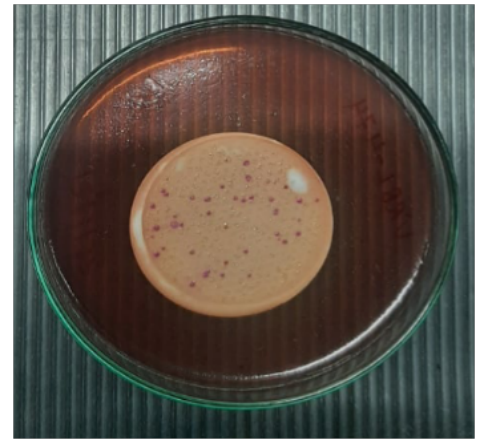
Coliforms on VRBL

.

Fecal streptococci on SBA

Enumeration of yeast and mold count (TPC & CYGA)

Detection of *Salmonella spp.*

Biochemical test of *salmonella spp*.

No.	Biochemical test	Reaction
1	TSI glucose (acid formation)	+
2	TSI glucose (gas formation)	+
3	TSI H_2S production	+
4	TSI lactose	-
5	TSI sucrose	-
6	L lysine decarboxylase	+
7	Indole	-
8	Voges- Proskauer	-

Detection of *Shigella dysenteria*   .   Biochemical test of *Shigella dysenteria*

No.	Biochemical test	Reaction
1	Catalase	+
2	TSI for H_2S	-
3	Urea	-
4	L- lysine decarboxylase	-
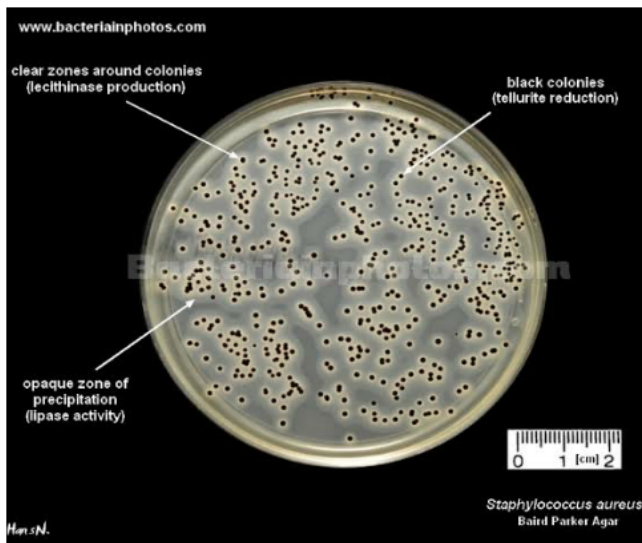
Detection of *S. aureus*   Detection of *Listeria monocytogens*
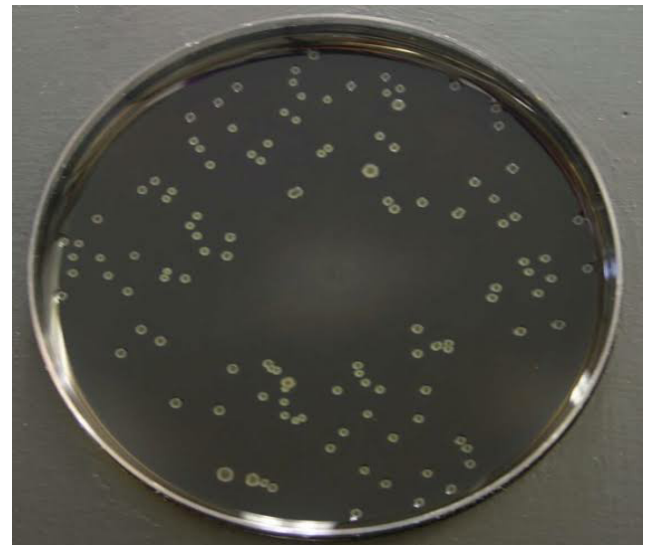
Conclusion

Food and water testing is most important thing because it have some harmful additives, low shelf life, some harmful pathogens or organisms are there. By doing this various testing we can detect the organisms which are harmful to human consumption. Various types of samples of food and water are analyzed and detect the organism if present. Microbiological testing is much important for some industries, pharmaceuticals, etc. By doing this internship I learned various techniques of microbiology.

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Reference

1. American Public Health Association. 1984. Compendium of Methods for the Microbiological Examination of Foods, 2nd ed. APHA, Washington, DC.
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6. Indian Standard (IS): 5887. (Part 3):1999
7. Indian Standard (IS): 5887 (Part 4):1999
8. Indian Standard (IS):14988 (Part 1): 2001
9. IS 5404:1984 and IS 5887:1999
10. IS 5404:1984 and IS 5402:2012

ANNEXURE 1

MacConkey Agar (MA)

Ingredients	Gms / Litre
Proteose peptone (meat and casein	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Sodium chloride	5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 gm
Agar	13.5 gm
Distilled Water	Add to make 1 Liter
Final pH (at 25°C)	7.3±0.2

Xylose lysine deoxycholate agar (XLDA)

Ingredient	Gms / Litre
Lactose	7.5 gm
Sucrose	7.5 gm
Sodium thiosulphate	6.8 gm
L- lysine	5.0 gm
Sodium chloride	5.0 gm
Xylose	3.75 gm
Yeast extract	3.0 gm
Sodium Deoxycholate	2.5 gm
Ferric ammonium citrate	0.8 gm
Phenol red	0.08 gm
Agar	15.0 gm
Final pH (at 25°C)	7.4 ±0.2

Rapport Vassiliadis salmonella enrichment broth (RVSEB)

ingredient	Gms/litre
Soya peptone	4.500
Sodium chloride	8.000
Dipotassium hydrogen	0.400

phosphate	
Potassium dihydrogen phosphate	0.600
Magnesium chloride,hexahydrate	29.000
Malachite green	0.036
pH after sterilization (at 25°C)	5.2 ± 0.2