

**“PURIFICATION AND DETECTIONS OF PATHOGENIC ORGANISMS
FROM MILK AND MILK PRODUCTS”**

An Industrial Training Report submitted

For the partial fulfilment of the Degree of Master of

Science by Pandhi Jilesh Jitendrabhai

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



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2022-2023

DECLARATION

I hereby declare that the work incorporated in the present internship report entitled **“PURIFICATION AND DETECTIONS OF PATHOGENIC ORGANISMS FROM MILK AND MILK PRODUCTS”**.

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 diploma.

Date:
07/04/2023

Jilesh Pandhi

Acknowledgement

Thanks God, to the merciful and the passionate, for providing us the opportunity to step in the excellent world of science. To be able to step strong and smooth in this way, we have also been supported and supervised by many people to would like to express our deepest gratitude.

The work was financially supported by **The Equity Laboratory-Rajkot**. The laboratory work was done in the microbiological testing laboratory.

After thanking God, who gave us the power to finish this work, we take this opportunity to express our sincere gratitude to **Mr.Divyesh Marviya** and his myriad contributes for our work and for patience, motivation, enthusiasm, and immense knowledge .His focused guidance help susduringthe writing of this project.

I also like to thank to our university authorities and **Dr.Nutan prakash visvakarma** Head of Biotechnology department. I am also very grateful to all faculty members, Department of microbiology for their encouragement and support throughout the period of the study.

Finally, I consider this as an opportunity to express my gratitude to all dignitaries who have been involved in successful completion of our project work.

Jilesh pandhi

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LIST OF ABBRIVATION

TPC	Total Plate count
PCA	Plate count agar
CYGA	Chloramphenicol yeast glucose agar
IS	Indian standard
D/W	Distilled water
RS-1	Reference stock -1
N-Saline	Normal saline
CFU	Colony Forming Unit
TNTC	Too numerous to count

ABSTRACT

Milk and milk products are the great source of nutrition for microbes. It is important to check quality of this milk, milk associated products and water before consumption because it has been provide opportunity to growth of pathogens and cause diseases in humans and animals. Total plates counts method and Yeast and mould detection and Enumeration method is used to determine Total number of microorganisms Which are present in the Milk and various products. By using IS:5887 (Part -1) Detection of *Escherichea coli* is done in to food stuffs and milk. Because *Ecoli* is the harmful bacteria to cause food poisoning in the humans. As per IS 5887 (part 3) Detection of *Salmonella* spp. into the cheese which is responsible for the severe disease in human. By using IS 5887 (part 2) Detection of *Staphylococcus aureus* in the milk and cheese. *S.aureus* is type of pathogen and it is cause the skin related infection (abscesses). By using FSSAI manual the detect the vanspati oil from the ghee and also check and confirm the purity of ghee. Also check the fat and protein content of milk. To preserve the bacterial culture also prepare the glycerol stock.

INTRODUCTION

➤ **NAME OF ORGANIZATION – THE EQUITY LABORATORY**

➤ **NATURE OF BUSINESS:**



1. Analytical Testing ,
2. Research & Development ,
3. Training ,
4. Services Provider.

➤ **Year of Established – August 2021**

➤ **Total number of Personnel – 06**

➤ **Type of Testing Services :**

1. Microbiological Testing Of Foods ,
2. Food & Agriculture Commodities Testing ,
3. Water And Soil Testing For Agricultural Work ,
4. Cosmetic , Pharmaceutical Testing ,
5. Snacks & Namkeen Testing

❖ INTRODUCTION

Milk and Milk Products:

Milk is a nutrient rich liquid food produced by the mammary glands of mammals. It is the primary source of the nutrition for young mammals including breast fed human infants before they are able to digest solid food.

Why milk is complete food:

It contains the protein, carbohydrates, all known vitamins, various minerals, and all the food ingredients considered essential for sustaining life and maintaining health.

Current scenario:

Milk is a very popular product for the adulteration and mixing purpose. It contains various nutrients which are responsible for the growth of pathogenic organisms. It causes various foodborne diseases. So, the milk and milk products require various microbial analyses to ensure the quality of the products.

1.Detection of *Escherichia coli* from Milk sample.

Aim: Detection and Enumeration of *Escherichia coli*.

Introduction:

This method are used to detect *E.coli* present or absent in sample. It is a qualitative method . *E.coli* is belong to Enterobacteriaceae family. *E.coli* is a Gram negative, rod shaped , facultative anaerobic coliform bacterium commonly found in intestine of human and animals.

Requirements:

- MacConkey's broth
- Eosin methylene blue agar
- Tergitol -7 agar
- MacConkey agar

Procedure:

Sample preparation

Take 200 ml peptone water and add 25 gram sample and mix it properly

Presumptive test

On day 2

Take 10 ml of Mac-Conkey broth add 1 ml of sample Incubate at 37°C for 24 hrs.

Confirmation test

Streak on EMB or Tergitol-7 agar or Mac-Conkey agar Incubate at 37°C for 24 hrs.

Positive Result:



EMB agar=pink colonies with green metallic sheen
Tergitol-7 agar = yellow colonies
MacConkey agar = pink colonies with precipitations



Gram's Staining

Bio-Chemical test

Gram negative and Indole = +Ve
rod shaped

Urease = -Ve
Methyl red = +Ve
TSI = +

RESULT : *E.Coli* & *Coliforms* are Presents in MILK sample.

Conclusion: The MILK is not safe for human or animal consumption



E.coli on MacConkey's & EMB Plates Respectively

2.Method for Enumeration of Total Bacterial Count .

Aim: Enumeration of Total Plate Count Method.

Introduction:

Milk products is highly susceptible to contaminated with various type of microorganisms along with this Other products like cheese, curd also contain different types of normal flora like E.coli .It is important to determine the number of microorganism which is present in this products, for this purpose we are use a total plate count method.

Principle:

Two poured plates are prepared using a specified culture medium and a specified quantity of the test sample, if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products. Other pairs of poured plates are prepared, under the same conditions, using decimal dilutions of the test sample or of the initial suspension. The plates are aerobically incubated at 30 °C for 72 hrs. The number of microorganisms per milliliter or per gram of sample is calculated from the number of colonies obtained on selected plates.

Materials:

1. Sample(Milk) ,
2. Plate count agar[PCA],
3. Spreader,

4. Laminar air flow,
5. Glasswares,
6. Isopropyl alcohol –Disinfectant,
7. Test Tubes for Dilution,
8. Distilled Water.

Media Preparation:

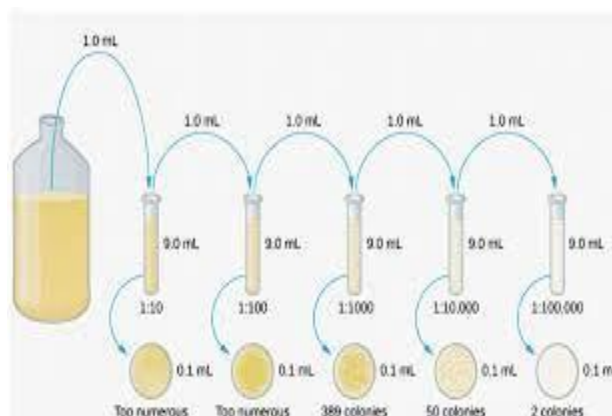
Weigh 3gm PCA (plate count agar) media for 100ml Add 2gm agar powder in 100 ml D/W water. Add 100 ml water and mix it properly Autoclaved the media at 121°C and 15 lbs pressure for 20minutes

Then pour the media in Petri dish under laminar air flow

Sample Preparation:

Take 10 gm Sample Add in 90 ml Sterile Distilled Water, [10⁻¹]

Mixed the sample . and perform the serial dilution respectively



Ten Fold Serial Dilution

Procedure:

After serial dilution take 0.1ml sample from 1:10 tube and spread into 10^{-1} PCA media plate Again take 0.1ml sample from 10^{-2} tube and spread into 10^{-2} PCA agar plate Same procedure is done till 10^{-5} dilution. Incubate plate at 30°C for 72 Hrs .Observed the result and then go for calculation.

Calculation: Count only those plates which ranges between 15 to 300 colonies

SET 1	SET 2
D1:TNTC(TOO NUMEROUS TO COUNT)	D1:TNTC(TOO NUMEROUS TO COUNT)
D2:219	D2:228
D3:17	D3:26
D4:4	D4:0

THE NUMBER OF BACTERIA PER GM OR ML SAMPLE IS EQUAL TO =

$$\Sigma c/[n1+0.1(n2)]d$$

Σc = Sum of all the colonies obtained from the countable plates.

n1= first countable dilution from set 1

n2= first countable dilution from the set 2

d= first countable dilution from the both sets.

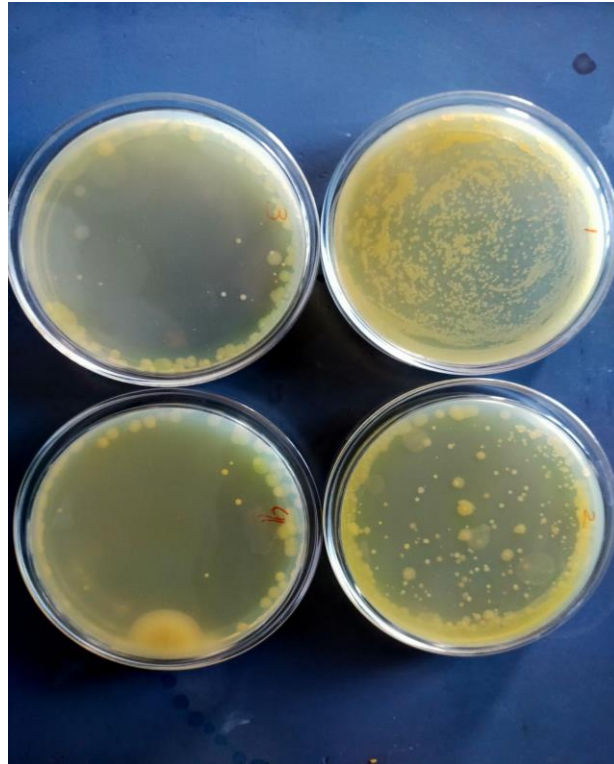
$$N = \Sigma c/[n1+0.1(n2)]d$$

$$490/2+0.1(2)(100)$$

$$490/0.022$$

$$22,272.727$$

$$2.2272 \times 10^4 \text{ CFU/ml}$$



Detection and Enumeration of bacterial count from Peanut sample

Result:

By performing this experiment the value of Bacterial enumeration to be found is 2.2272×10^4 CFU / ml.

3.ENUMERATION OF TOTAL YEAST AND MOULD COUNT:

Total yeast and mould count that determine the total amount of viable yeast and mould present in the sample. Total yeast and mould count used to detect and quantify the fungal growth.

Media:

Chloramphenicol yeast extract glucose agar

Procedure:

Spread plate method

Take 90ml sterile D/W and Add the sample in sterile D/W (if the sample is solid than take 10gm ,if the sample is liquid than take 10ml).Thoroughly mix all the components properly. Prepare the 10fold serial dilution upto 4 or 5 steps and after the serial dilution the take 0.1 ml solution of each dilution tubes and spread on the chloramphenicol yeast extract glucose agar media plates respectively. Incubates all the plates at 25C for 5 days.

Pour plate method

Take 90ml Sterile D/W, Add the sample in sterile D/W (if the sample is solid than take 10gm ,if the sample is liquid than take 10ml).Thoroughly mix all the components properly. Prepare the 10fold serial dilution upto 4 or 5 steps. Take and add 1ml of sample in sterile empty petri plate. Pour the chloramphenicol yeast extract glucose agar into each plates approximately 12 to 15 ml and allow it to solidify. Again pour the media and allow to solidify after that incubate all the plates at 25 C for 5 days.

Calculation and Result

After incubation count the colonies on the plates .
Countable Range : 15 to 150 colonies consider for calculation

Calculation:

$$N = \frac{\sum C}{(n_1 + 0.1n_2)d}$$

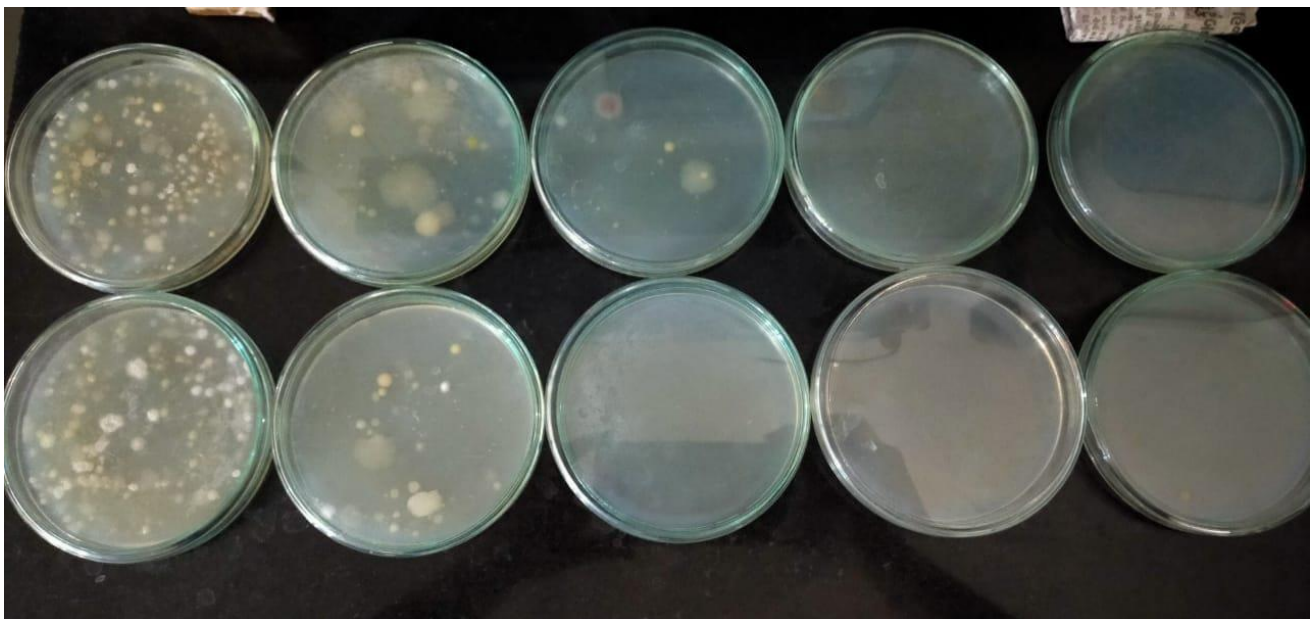
$\sum C$ = the sum of the characteristic colonies counted on all the dishes retained.

n_1 = the number of dishes retained in the first dilution

n_2 = the number of dishes retained in the second dilution

d = the dilution factor corresponding to the first dilution

Report count as colony forming unit/gram or ml



Yeast and Mould detection and enumeration set-1 and set-2

Result : the result of milk sample can be consider in the range of yeast and mould count according to IS 5403

Conclusion : The milk sample is consumable for human health.

4 .ANALYSIS OF MILK BY MILK ANALYSER

Milk analyser is one type of instrument, by help of them we can analysed the fat, solid not fat (SNF), water, protein, lactose, salt.

Principle:

Milk analyser work on the principle of non-destructive ultrasonic technology. Milk analyser make fast analyse of milk major composition like fat, solid not fat (SNF), added water, protein, lactose, salt in percentage [%] and temperature of milk. The sample to be used are raw milk without any processing.

Procedure:

Preparing milk sample for analysis: The most important requirement for the milk sample is, it should be homogeneous free from air bubbles and temperature of the milk should be between 10°C to 40°C.

Milk sample stirrer by help of digital ultrasonic stirrer (11sec) the switch of milk analyser(Warming up for few minutes).

First give rinse with water, Then select option for which type of milk analyse(Buffalo milk, cow milk, mix milk), Then fill up the container with test sample of milk Put container for analysis.

After approximately 38 seconds result shown on display.

If we want to analyse second milk sample then it's necessary to give two times water wash before analysing second sample.



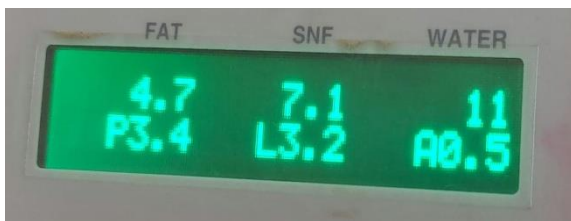
Milk Analyser



Digital ultrasonic Stirrer

Parameter	Range
Fat	0.5 ~ 15 %
SNF	3 ~ 15 %
Added Water	0 ~ 100 %
CLR	20 ~ 40
Lactose	1 ~ 8 %
Protein	1 ~ 6 %
Salt	0.2 ~ 1.5 %

Standard range of Milk



LED display

5. DETECTION OF VARIOUS ADULTERANTS FROM THE MILK

❖ INTRODUCTION:

Milk adulteration test kit from NICE chemicals private limited help to detect adulterants like hydrogen peroxide, urea, starch, neutralizer, detergents, glucose-dextrose, sodium chloride, acidity, mastitis, formaldehyde, maltose dextrin, nitrate-nitrogen from milk samples.

Why adulteration of milk?

Unfortunately milk is being very easily adulterated throughout the world. Possible reason behind it may include Demand and supply gap, Perishable nature of milk, Increase the volume of milk for better prices.

1. Detection of urea

Take 2 ml of milk Sample In test tubes and add 2ml of urea and reagent 1 And mix it.

Observation:

Normal pure milk: Slight yellow color

Adulterated milk: Distinct or pale yellow color

2. Detection of Starch

Take 3ml of milk in test tubes. Boils for few minutes and add 3 drops of starch Reagent 1 and mix it.

Observation:

Normal pure milk: no color change

Adulterated milk: Blue color

3. Detection of Neutralizer

Take 5ml of milk sample in test tube. Add 4 drops of neutralizer reagent 1
Mix it properly

Observation:

Normal pure milk: Pale yellow color change

Adulterated milk: Dark purple color

4. Detection of Sugar

Take 5ml milk and Add 2ml of sugar Reagent 1 and add 4 drops of sugar
Reagent 2 and mix It properly and put in boiling water bath For 2 minutes.

Observation:

Normal pure milk: Light brown color

Adulterated milk: Red color

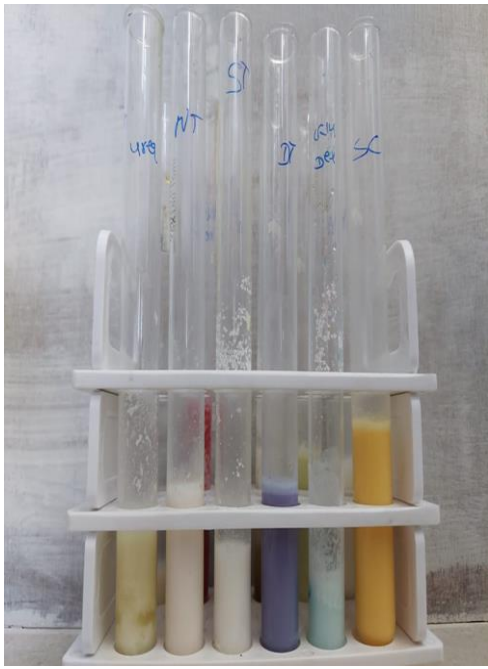
5. Detection of glucose

Take 1ml milk and add 1ml glucose-dextrose reagent 1 and mix it and
boils for 3 minutes. cool down the tubes and add 1ml of glucose reagent 2.

Observation:

Normal pure milk: Light blue color

Adulterated milk: Dark Blue color



Milk adulteration test result

6 . Preparation of Glycerol stock

Aim: To Maintain the Organism via glycerol solutions.

Principle: A Glycerol stock is type of suspension used to store bacterial cultures over long periods of time in laboratory settings. When liquid bacterial culture is added to 50% glycerol solution, the glycerol enters the bacterial cells, rendering them structurally stable and allowing them to be stored safely.

Requirements:

0.86% Normal Saline,
Glycerol solution,
Double distilled water,
Screw cap bottle,
Cryophills vials,
Petri plates,
Safety Items,

Master Stock

Master stock preserve for 1-2 years



This process done into 10 second



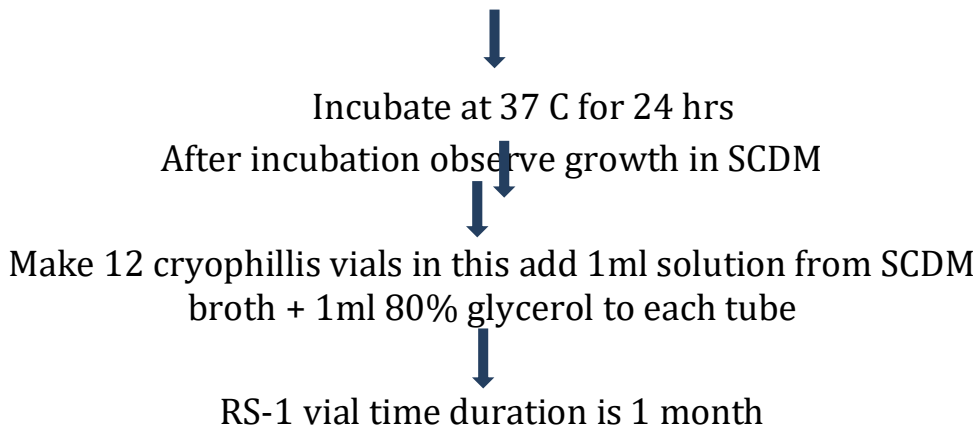
Transfer 1ml suspension to 4 cryophillis vials and add 1ml 80% glycerol solution and mix it properly



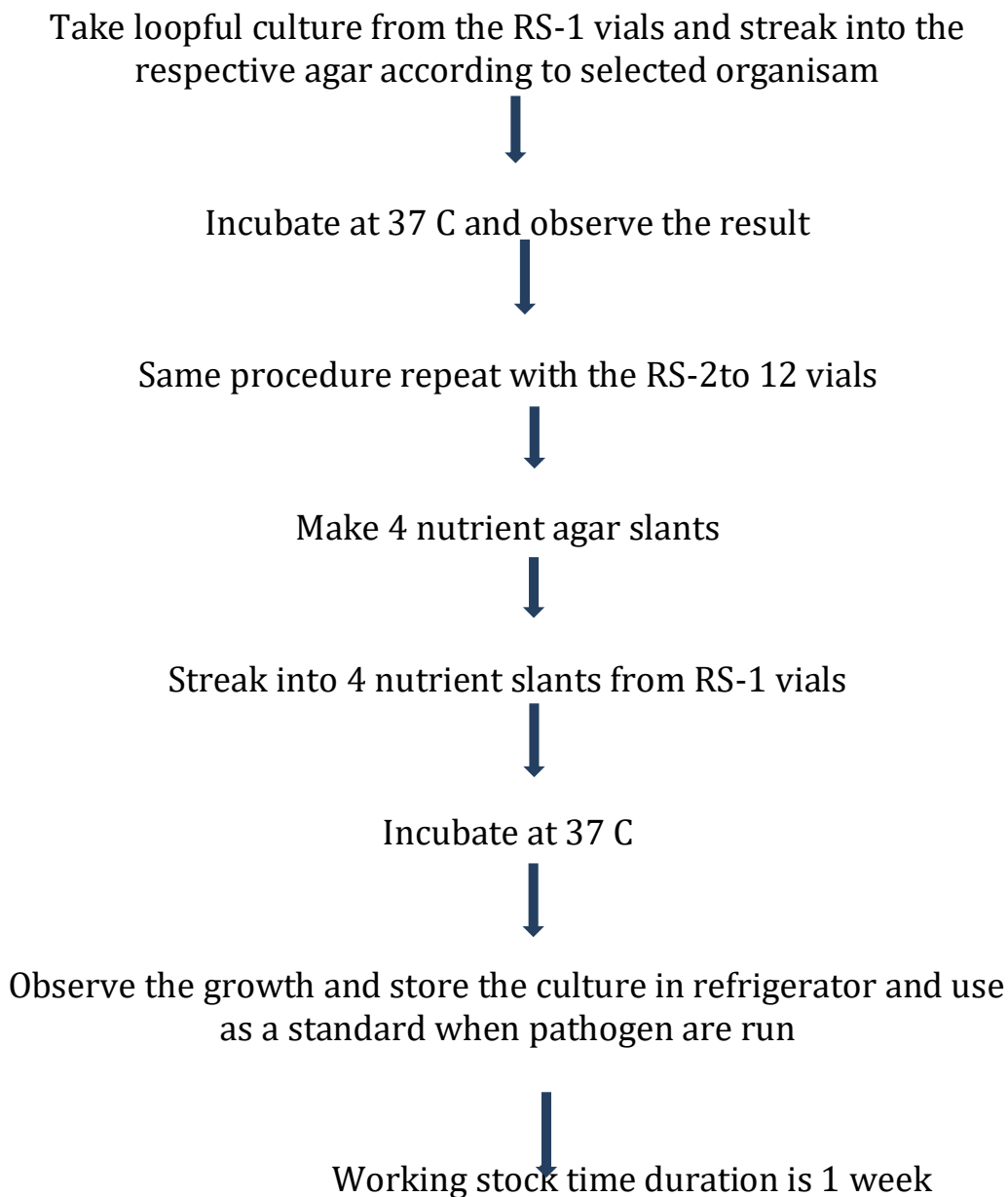
Master stock preserve for 1 to 2 years

Reference stock

Take 1ml solution from N-saline + Bacterial powder suspension and add into soya bean casein digest medium



Working stock



7. REFERENCES

IS 5403:1999 -- Yeast and mould count of foodstuffs ,

IS 5402: 2012 -- Total plate count(TPC) ,

IS 5887(:Part-I) – 1976 –Method for Detection and Enumeration of bacteria
Responsible for food Poisoning,