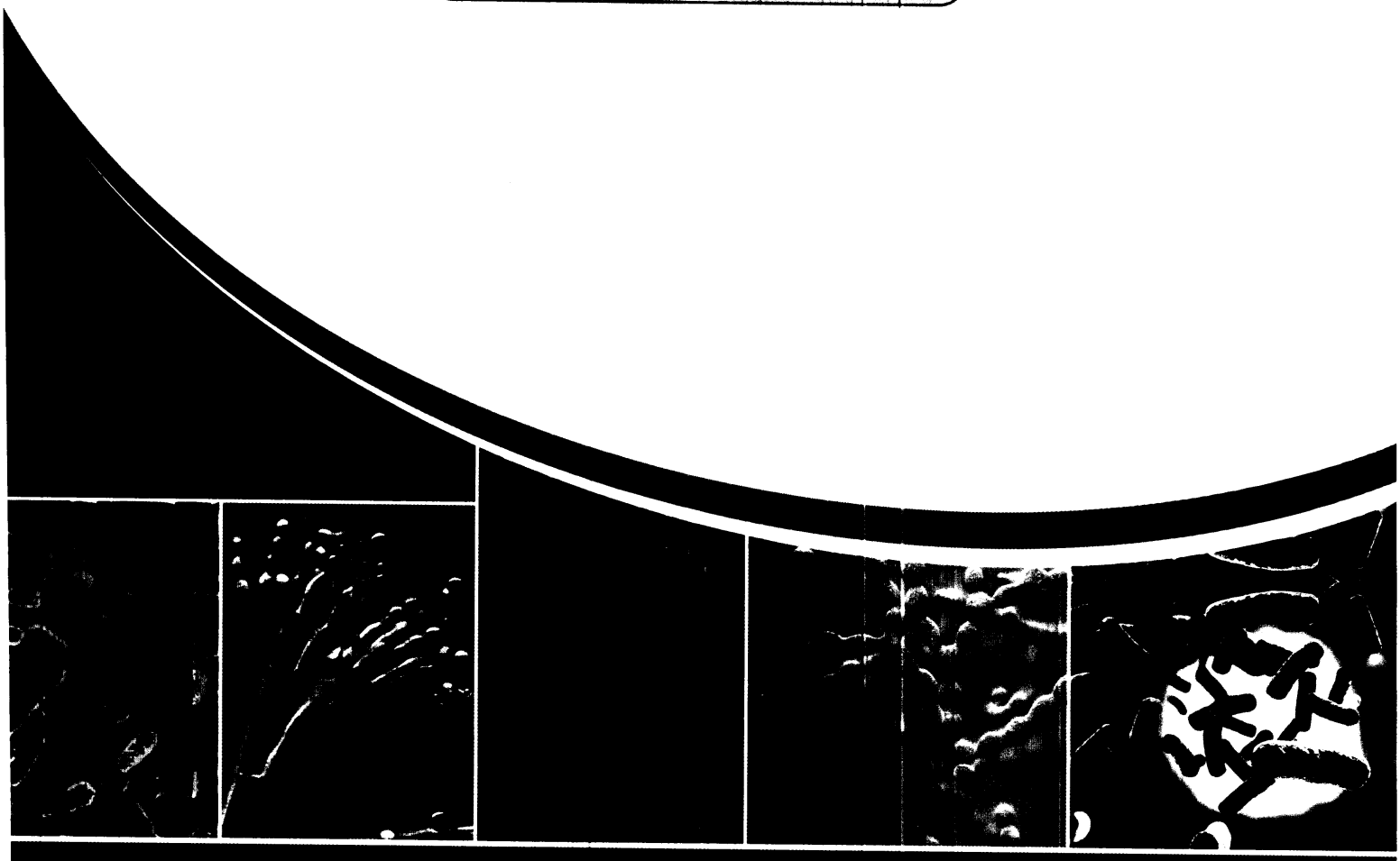
PRACTICAL MANUAL

Food Microbiology

B. Tech. (Food Technology)

Semester : II
Course No.: FMS- 122



Department of Food Microbiology and Safety
College of Food Technology
Vasantrao Naik Marathwada Krishi Vidyapeeth
Parbhani-431 402

Prepared by

Prof. H.W. Deshpande
G.M. Machewad
Department of Food Microbiology and Safety
College of Food Technology, VNMKV, Parbhani

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Associate Dean and Principal
College of Food Technology
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani

Department of Food Microbiology & Safety
College of Food Technology
VNMKV, Parbhani

Certificate

This is to certify that Mr/Miss _____
Studying in B.Tech (Food Tech) II[nd] semester has performed set of Practicals of the course subject **Food Microbiology** with course number FMS-122 satisfying in the year 20 - 20

Date:

Course Teacher

Head
Deptt. of FMS

INDEX

Isolation of Yeast from starchy Food sample

INTRODUCTION :

- Yeast (Saccharomyces cerevisiae var. ellipsooidens) is found in starch and sugar containing substrates such as flour, bakery products (bread), sugar syrup, etc.
- They may be isolated by direct plate method and dilution plate method.

REQUIREMENTS:

Materials: Flour or other sample, YPS Medium

Glassware and Equipment: Sterile Petri plates, Autoclave, Sterile flasks, Incubator

YPS Medium

Soluble starch	5.0 g
Yeast extract	5.0 g
K_2HP_4	1.2 g
MgSO4	0.5 g
Agar	20 g
Sterile Distilled water	1 Litre
pH	3 – 3.5
Chloramphenicol	70 mg (to be added after autoclaving)

PROCEDURE:

1. Collect the sample and mix with sterile distilled water in the ratio of 1:10 (w/v).
2. Dispense 1 ml aliquot suspension into sterilized petri dishes counting 10-15 ml of YPS media
3. Incubate the plates at 28°C for a few days.
4. Appearance of small colonies indicates the presence of yeast.
5. Identify yeast microscopically on the basis of vegetative cells, ascospores, other structures such as budding cells.

OBSERVATIONS :

Sr.No.	Microscope observation	Presence/ Absence (description)
1	Vegetable cells	
2	Ascospores	
Other observations		
3		
4		
5		
6		

CONCLUSION:

Isolation of and Enumeration of Molds from Food Sample

INTRODUCTION:

- A large number of fungi of different groups are found in food.
- The food-borne fungi can be isolated from bread and their total population enumerated following the method given below:

REQUIREMENTS:

Materials: food sample,

Glasswares and Equipments: pipettes, incubator, cotton plug, Autoclave, microscope, cotton blue + lactophenol, etc.

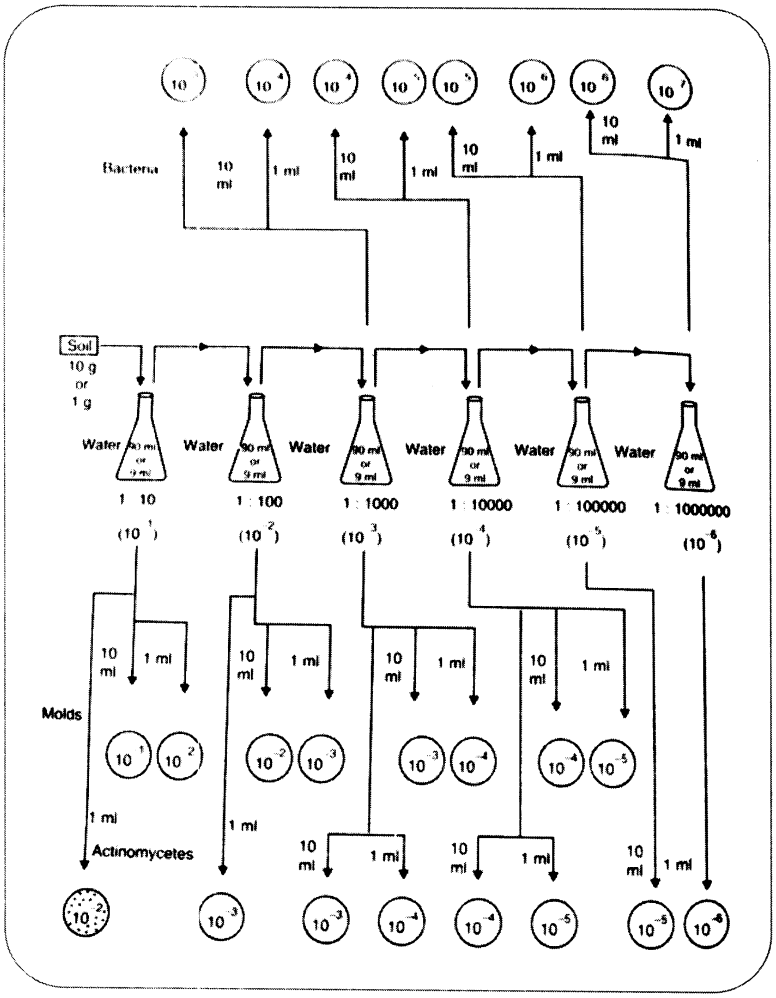
Chemicals: Penicillin 30 mg (to be mixed well with autoclaved medium just before pouring), Streptomycin 30 mg (to be mixed as penicillin).

Martin's Agar (Peptone – Dextrose – Rose Bengal Agar Medium):

Agar	20 g
KH_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Pentone	5.0 g
Dextrose	10 g
Distilled water	1 Litre
Rose Bengal (1%)	3.3 ml
Streptomycin	(30 mg) to be added after autoclaving and just before pouring)

PROCEDURE:

1. Take 3 flasks (250 ml capacity), transfer 90 ml distilled water in each flask, plug them properly, label 1 – 3 and autoclave at 15 lb/inch^2 for 30 minutes.
2. Collect small amount of sample from 5 different foods and mixed to make on lot.
3. Weight 10 g of sample and transfer into flask 1 containing 90 ml sterilized water. It gives homogenous food suspension.
4. Transfer 10 ml of food suspension from 10^{-1} dilution into flask 2 containing 90 ml sterilized water to get dilution 10^{-2}. Mix the suspension gently.
5. Similarly serially transfer 10ml food suspension from 10-2 dilution into flask 3 containing 90 ml water to get the final dilution of 10^{-3}. Mix the suspension gently.
6. Aseptically pour 1 ml soil suspension from10^{-3} dilution into martin's agar plates (supplemented with both streptomycin and penicillin at the rate of 30 mg/L each).



7. Gently rotate the plates so as to spread the suspension on medium).
8. Incubate the plates at 25°C for 4 -- 5 days.
9. Transfer 1 ml food suspension from 10^{-5} dilution into Thornton's agar plates in three replicates each containing 30 mg/L Nystatin (supplemented after autoclaving just before pouring into plates.
10. Gently shake the plate so as to spread suspension uniformly on the medium

OBSERVATIONS:

Fungal colonies of different size and colour growth on medium. For qualitative study peck up small amount of mycelia growth with sterile needle, transfer on glass slide containing 1 drop of cotton blue plus lactophenol, observe under microscope and identify them following mycological literature. Measure the occurrence of each fungal species by uising formula.

$$\text{Occurrence (\%)} = \frac{\text{Average number of colonies of a species}}{\text{Avg. No. of colonies of all fungal species}} \times 100$$

RESULTS:
Fungal species

Sr. No.	Morphological Description	Fugal species	% Occurrence
1			
2			
3			
4			
5			

QUANTITATIVE STUDY

1. Take 10 g of food sample from the same lot, transfer in pre-weighed beaker (100 ml capacity) and put it in oven.
2. Make the food sample oven dry at 85°C for 24 hrs. count all the colony forming units (CFUs), take average three plates and calculate average number of CFU/g dry sample by using formula.

$$\text{Colony forming units (CFU/g) dry food} = \frac{\text{Average number of colonies}}{\text{Dry weight of sample}} \times \text{dilution factor}$$

N.B.: If number of colonies are innumerable and difficult to count, further dilute the sample and plate it as done before.

OBSERVATIONS:

Sr. No.	Mold growth	CFU/g
1		
2		
3		

CONCLUSION:

Isolation of Bacteria from Food Sample

REQUIREMENTS:

Materials: food sample,

Glasswares and Equipments: As described in Practical No. 2.

Thornton's Standardized Agar (Thornton, 1922)

Agar	15.0g
H_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	0.1
NaCl	0.1g
$FeCl_3$	trace
KNO_3	0.5 g
Asparagine	0.5g
Mannitol	1.0 g
Distilled water	1 Litre

PROCEDURE:

1. Take 3 different food samples.
2. Take 5 flasks, transfer 90 ml water in each, ploug them, label 1 – 5 and autoclave.
3. Serially make dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.
4. Transfer 1 ml food suspension from 10^{-5} dilution into Thornton's agar plates in three replicates each containing 30 mg/l Nystatin (supplemented after autoclaving just before pouring into plates.
5. Gently shake the plate so as to spread suspension uniformly on the medium
6. Incubate the plates at 25+1oC for 24 to 48 hours.
7. Pick up each colony, prepare smear and perform Gram's staining for differentiation.
8. Count CFUs of bacteria.
9. However, if specific bacteria of interest present, isolate it aseptically and perform further bacteriological tests for identification.

OBSERVATIONS :
Viable Plate Count of Bacteria

Sr. No.	Particulars	Bacterial Count (CFU)
1	Viable Plate Count (Sample – 1)	
2	Viable Plate Count (Sample – 2)	
3	Viable Plate Count (Sample – 3)	

CONCLUSION:

Microbial Examination of Cereal and Cereal Products (Bread)

INTRODUCTIONS :

These food products usually do not support growth of bacteria but cream and other fillings of cakes are highly favourable for multiplication of bacteria very rarely species of bacillus may cause defect in bread known as ropiness due to production of capsular material

The material in microbiological examintion of cake filling depend upon nature of constituents and are given in the sections appropriet to constituent. Microbiological control of bakery products other than filling is concerned mainly with possiblity of spoilage due to growth molds since the bakery temperature is sufficient to kill fungal spores subsequent spoliage usually caused by mold contamination from the almosphere or from wrapping material.

REQUIRMENTS :

- Bread Sample
- Sterile Petriplates.
- Lacto phenol cotton blue solution
- Glass slides and cover slipes.

PROCEDURE :

Moistened bread sample was kept in sterile petriplates

- it was incubated at room temperature for 5 days.
- microscopic observation of different molds was carried out.

RESULT :

CONCULSION :

The microflora carried by bread consisit of penicillium, As per gillus, Mucor, Rhizopus and some bluish green spots.

Microbiological Exmination of Fruits and Vegetables.

INTRODUCTION :

Fresh fruit and vegetables normally carry a surface flora of micro-organisms some of them play role in spoilage. Microbiological examination of these foods is not easily carried out. But in case of fruits and vegetable handling is involved eg salad preparation an exmination of contamination is advisible

EXAMINATION OF POTATO & TOMATO

The interior part of the intact healthy fresh fruit and vegetables are usually sterile there fore such tissue removed aseptically can be used as culture media without heat treatment in incubation shows tissues to be setrile. Since skin of most vegetables and fruits eg. Tomato contains all microganisms present count of organisms on such vegerables should be experesed in term of area of skin exmination.

REQUITMENTS :

- Tomato and potato
- Sterile knife
- Setile mortr & sand
- Serile buffered sistilled water.
- Sterile petriplates and pipetts.
- 95 % ethanol
- Plate count broth and agar.
- Brilliant green lactose bile broth.

COMPOSITION

Peptone ..... 10 gms
Lactose..... 10 gms
Sodium taurocholate..... 20 gms
Sodium Chloride..... 5 gms
Distilled water ..... 1 lit.
pH..... 7.5

All ingrediants were dissolved and 1.3 % brilliant green [aqueous solution – 1 ml] was added Durhams tube were put in test tube and medium was strized at 110oC for 40 min.
- Sabouraudos agar.

Composition :

Gulcose..... 40 g
Peptone..... 10 g
Agar..... 18 g
Distilled water ..... 1 lit.
pH..... 6.8 to 7

Sterize by autoclaving at 110oC for 10 minutes.

A1 PLATING OF SURFACE OF TOMATO

PROCEDURE :

- 5 cm square area of skin and adjonining flesh was cut tith a shart knife
- it was grounded sand and tissues were transfer to 99 ml of buffered distilled water and shaken vigously
- dilutions were prepared (1:10, 1:100, 1:1000)
- Duplicate plates were prepared for each dilution on plate ount agar.
- The plates were incubated at room temperature for 5 dsys or at 30oC for 2 days
- In the same way plates were poured with sabouradus agar for yeast and molds and incubated at room temperature for 2 days or 5 days as required.

Results :

II TEST FOR STRILITY OF INNER TISSUE

REQUIRMENTS :

- Tomato
- 95 % ehnanol
- sterile petriplates & pipetts.
- plate count broth.
- Brillant green lactose bile broth.

PROCEDURE :

- One side of well sound tomato was wetted with 95 % ethnol, burned off latter and throughly flammed.
- The flammed skin was piericed with setile end of pipette and 3 ml of juice and pulp was drawn out.
- 1ml of juice and pulp was blown in each of 2 tubes of plate count broth.
- Tubes were inculabed at room temperature for 5 days or 30 to 32oC for 2 days and then it was observed for growth.
- Similary double and single strength tubes of BGLBB were inoculated with dilutions prepated from tomato juice and pulp inorder to calculate MPN

RESULTS :

CONCLUSION : This shows that the inner fisseus of tomato were not sterile.

Mictobial Exmination of Meat

INTRODUCTION :

The muscle tissue of healthy animal contains a few bacteria but becomes easily contaminated after after slaughtering and during and after preservation. Bacteria can readly multiply on the cut surface although the bacterial count of interior of the meat usually remains much lower bacteria which may be present include Micrococci, Achromobactor, Pseudomonas and also Staphylococci, Lactobacilli, Sterptococcus, Brucella, Salmonella, Mycobacterium. Mold spoliage of cut surfaces refrigerated or frogen meat can occur at temp. down to about -5°C. At the lower tempereature mycelial growth may occur without spore production and this will give rise to a thin fluffy appecuence caused by for eg. Mucor Rhizous other types of mold sopliage which may be obivious on inspection are "White Spots" caused by eg. Penicillium. In addition ceretain yeast may cause low temperature spoilage.

Surgace slime on meat is usally caused by Pseudomonas, Achromobactor, Streptococcus, Lactobacillus, leuconostoc, Bacillus and Micrococcus may also Putrefaction in addition to the members of the genus Clostridia. The lactic acid bacteria on the other hand can cause souring due to the production of organic acid.

The bacerial condition of the meat is determined by taking both superficial and deep tissue samples. The microboligical exmination of surface is best carried out by taking superfical samples as very thin slices using sertile scalpels and forceps. Thses samples are homogenised in a diluent to give 1:10 dicution deep tissue samples should also be taken separtely.

REQUITMENTS :

- Meat sample
- Plate count agar
- Violet red bile agar
- Lauryl tryptose broth
- Reinforced closteridial meat medium composition
- Yeastrels yeast extract.....3 gm
- Peptone.....10 gm
- Lab lemco meat extract.....10 gm
- Peptone ..... 10 gm
- lab lemcomeat extract .....10 gm
- D.Glucose ..... 5 gm
- Sodium acetate (Hydrated) ... 5 gm
- Cyslein ..... 0.5 gm
- soluble starch ..... 1 gm
- agar ..... 5 gm

-Distilled water ..... 1 ltr.

-pH..... 7.1 to 7.2

Add all the ingredients, heat in untill dissolved and then filter through a hot paper pulp adjust to pH 7.4 dispense in screw cap tubes and sterize by autoclaving at 121oC for 15 minute.
- sterile petriplates and flasks.

PROCEDURE :

10 gm of meat sample was weighed aseptically and added into 90 ml of sterile distiller water diluent and was homogenised using sterile blender this gives initial 1:10 dilution form this serial dilutions up to 5 were prepared 1 ml of each dilution was then pipetted out in to a suitable medium for a perticular group of organisms as follows.

Plate count technique : plates were poured with plate count agar and were incubated with at 5°C for days 25°C for 3 days and 37°C for 48 hrs.

Presumptive coliform count

In order to detect the colifor count pour plate was carried out using violetred bile agar and the plated were incubated at 35 °C presumptive coilform count using laurl tryptose broth dilution tube technique using laurl tryptose broth tube incubation at 37°C for 24 hrs.
Dilution tube technique using laurl tryptose broth was carried out in order to detect if very low number a coliforms were present the tubes were incubated at 85°C for 48 hrs.

After 48 hrs positive reaction tubes were counted and MPN was General Viable count

Anaerobic Bacteria

- 10 gm of meat sample was weighed aseptically and added in to serile reinforced clostridial medium used as diluen. Form this fooet her serial dilutions were prepated.
- 1 ml of each dilutions were pipetted out in to steile petriplate and then pouted with plate count agar.
- after soldification plates were placed in anaeribic jar and incubated at 37°C for 48 hrs.

RESULTS :

CONCLUSION :

Microbiological Examination of Egg.

Shell eggs :

The contents of greater majority of new laid eggs are sterile. The outside of the shell normally carriers large number of bacteria as a result of contamination of the egg shells as a result of faeces. These bacteria are grain negative rods including species of pseudom monas, Alcaligens , Achromobactor, proleus tero bactor escherichia flavobacterium and also some Bacillus species. Cocci and moulds rough handling or washing of egg instedot dry cleaning will allow penetration of the shell by the surface contamination and therefore a microbial examination of shell egg may some thims be required

REQUIRMENTS :

- Egg
- A spoon
- Physiological saline (0.85 % Nacl)
- Plate count agear
- Lauryl tryptose broth composition

Tryptose..... 2.09 ms.
KH_2PO_4..... 2.75 gms.
K_2HPo_4..... 2.55 gms.
Nacl..... 5 gms
Lactose..... 5 gms.
Sodium lauryl sulfale.... 0.1 gms
Distilled water ..... 1000 ml.

Sterlization the medium by autoclaving at 121°C for 15 Minute
Terathionate Broth

Solution A:

Sodium thiosulfate (Hydrated)..... 24.8 gm
Distilled water ..... 100 ml

Sterize by steaming on each of three sucessive three days

Solution B :

Iodine ..... 12.7 gm
Potassium Iodide .....20 gms

Preparation of complete medium :

To 75 ml of nutrient broth add 2.5 gms of $CaCo_3$ and sterlige by steaming for 30 minute on each of the three successive days. When cooled at 15 ml of sol2 A and 4 ml of solution B Distribute aseptically in to sterile container and store in referigerator. This medium can be stored Dor one week but the components solution A, Solution B and Sterized chop nutrient broth may be conveniently stored seperately untill immedialety become use.

Violet Red Bile agar -

Preparation of egg sample. B

- The egg was scrubbed with warm, soapy water, with a SHDD brush.
- Then it was rinsed with and dried and immersed in alcohol for 10 minutes and it was drained well and flamed quickly.
- A hole was cut in the end opposite the air sac which was located at the blunt end using a sterile scalpe
- The contents were removed aseptically and home genised using a food mixer.
- 10 gm of blended egg was weighed out in to sertile wide mouth glass bottle containing 90 ml diluent.
- From these initial serial dilutios were prepared in usual way.

PROCEDURE :

- Plate counts on place count agar were carred out at 20 oC, 25oC and 37oC and presumptive coil dorm counts using either Laury tryptose broth or violet red bile agar duck egg can be vehicle for salmonella.
- Before blending 1ml portion of the albumin and yolk was taken and transfered in to duplicate tubes of sterile broth incubated at 37oC for 2 days it was exminaed microscopically and macroscopically for evidence of growth and recorded as positive and negative results.

A. General viable count :

- ml of each 1 : 100, 1:1000 and 1:10000 dilutions was plated out
- Pour plate technique was carried out using plate count agar.
- Plates were incubated at 30oC for 3 days.

Result : (Numerous uncountable colonies were present - on the plates after incubationg at 30oC for three days)

B. Coliform Count [MPN]

- The dilution of egg sample were inoculated in lauryl trypotse broth for caliform count.
- 10 ml 1:100 dilution was added to a set of 5 tubes containing 10 ml lauryl tryptose broth.
- Then 1 ml and 0.1 ml of 1:100 dilution was added to a set of 5 tubes containg lauryl tryptose broth.
- The tubes were incubated at 87oC for 24 hrs
- on the other hand pour plate technique was carried out using violetred bile agar.
- Plates were incubated at 37oC for 24 to 48 hrs

Results :

C – Presence of Salmonella in egg sampe. B

- 10 ml of blened yolk was added to a flask containing 90 ml of treathionate broth
- The both medium was incubated at 37oC for 24 hrs.

Result :

D - Psychrophilic plate count :

- 1 ml of 1:100, 1:1000 and 1:10000 dilution were pipetted out in a sterile petriplates.
- Then plate count agar was poired by pour plate technique.
- Plate were incubaed at 5 to 7°C for 7 days

Result : No colonies appered after incubating for 15 days at 5°C

RESULTS :

A]

B]

C]

D]

CONCLUSION :

The inetrior of the egg was contaminated with the bacteria.

PRACTICAL NO. 8

Quantitative Analysis of Milk by Standard Plate Count Method

INTRODUCTION:

Milk is important source of transmission of various pathogenic microorganisms in human beings. Milk provides an excellent growth media for bacteria at room temperature. Dairy produces may be contaminated either by soiled hands of workers, unsanitary utensils, flies, and use of polluted water. Sanitary quality of milk can be checked by bacterial count in milk. Similarly, improper handling of foods spoiled in the home, hospitals, institutions, canteen, etc results in spread of pathogens.

Quantitative analysis of milk by SPC Method

Standard Plate Count is considered as standard method for quality check of milk. Presence of human pathogenic bacteria may arise due to unsanitary handling of milk, diseased udder or improper storage conditions. The increase in number of bacterial count has the more possibility of transmission of disease.

MATERIALS REQUIRED:

Materials: Milk sample, Nutrient agar, distilled water

Glasswares and equipments: petri plates, pipettes, incubator, colony counter, etc.

PROCEDURE:

1. Collect raw milk sample
2. Dilute the sample of milk in sterile distilled water for isolation of microorganisms.
3. Transfer 1 ml of milk serially in sterile distilled water to make dilution: 1: 100, 1: 10,000 and 1: 10,00,000.
4. Transfer 0.1 ml and 1 ml of the milk from each diluted sample bottle into two separate petri plates and pour sterile nutrient agar medium into each plate.
5. After the nutrient agar has solidified, incubate the plates at 35oC for 24 to 48 hours.
6. As stated in serial dilution technique, count the plates containing 30 to 300 colonies for calculating number of organisms per milliliter of the original milk sample.
7. Prepare a smear of the milk film and observe for presence of bacteria.

The microscopic appearance give type and arrangement of bacterial cells, count the numbers of bacterial colonies per petri dishes, take average of three plates and estimate CFTs per ml of milk.

CALCULATIONS:

OBSERVATIONS :

Sr. No.	TPC	CFU/g
1		
2		
3		

CONCLUSION:

Microbial Examination of Canned Foods

INTRODUCTION:

The incidence of spoilage in canned foods is low, but when it occurs it must be investigated properly. Swollen cans often indicate a spoiled product. During spoilage, cans may progress from normal to flipper, to springer, to soft swell, to hard swell. However, spoilage is not the only cause of abnormal cans. Overfilling, buckling, denting, or closing while cool may also be responsible. Microbial spoilage and hydrogen, produced by the interaction of acids in the food product with the metals of the can, are the principal causes of swelling. High summer temperatures and high altitudes may also increase the degree of swelling. Some microorganisms that grow in canned foods, however, do not produce gas and therefore cause no abnormal appearance of the can; nevertheless, they cause spoilage of the product.

Spoilage is usually caused by growth of microorganisms following leakage or under processing. Leakage occurs from can defects, punctures, or rough handling. Contaminated cooling water sometimes leaks to the interior through pinholes or poor seams and introduces bacteria that cause spoilage. A viable mixed microflora of bacterial rods and cocci is indicative of leakage, which may usually be confirmed by can examination. Underprocessing may be caused by undercooking; retort operations that are faulty because of inaccurate or improperly functioning thermometers, gauges, or controls; excessive contamination of the product for which normally adequate processes are insufficient; changes in formulation or handling of the product that result in a more viscous product or tighter packing in the container, with consequent lengthening of the heat penetration time; or, sometimes, accidental bypassing of the retort operation altogether. When the can contains a spoiled product and no viable microorganisms, spoilage may have occurred before processing or the microorganisms causing the spoilage may have died during storage.

Underprocessed and leaking cans are of major concern and both pose potential health hazards. However, before a decision can be made regarding the potential health hazard of a low-acid canned food, certain basic information is necessary. Naturally, if Clostridium botulinum (spores, toxin, or both) is found, the hazard is obvious. Intact cans that contain only mesophilic, Gram-positive, sporeforming rods should be considered underprocessed, unless proved otherwise. It must be determined that the can is intact (commercially acceptable seams and no microleaks) and that other factors that may lead to underprocessing, such as drained weight and product formulation, have been evaluated.

The preferred type of tool for can content examination is a bacteriological can opener consisting of a puncturing device at the end of a metal rod mounted with a sliding triangular blade that is held in place by a set screw. The advantage over other types of openers is that it does no damage to the double seam and therefore will not interfere with subsequent seam examination of the can.

Useful descriptive terms for canned food analysis

<table>
<tr><td colspan="2"><u>Exterior can condition</u>
leaker
dented
rusted
buckled
paneled
bulge</td><td colspan="2"><u>Internal can condition</u>
normal
peeling
slight, moderate or severe etching
slight, moderate or severe blackening
slight, moderate or severe rusting
mechanical damage</td></tr>
<tr><td colspan="2"><u>Micro-leak test</u>
packer seam
side panel
side seam
cut code
pinhole</td><td><u>Product odor</u>
putrid
acidic
butyric
metallic
sour
cheesy
fermented
musty
sweet
fecal
sulfur
off-odor</td><td><u>Product liquor</u>
cloudy
clear
foreign
frothy</td></tr>
<tr><td><u>Solid product</u>
digested
softened
curdled
uncooked
overcooked</td><td><u>Liquid product</u>
cloudy
clear
foreign
frothy</td><td><u>Pigment</u>
darkened
light
changed</td><td><u>Consistency</u>
slimy
fluid
viscous
ropy</td></tr>
<tr><td colspan="4">Flat - a can with both ends concave; it remains in this condition even when the can is brought down sharply on its end on a solid, flat surface.</td></tr>
<tr><td colspan="4">Flipper - a can that normally appears flat; when brought down sharply on its end on a flat surface, one end flips out. When pressure is applied to this end, it flips in again and the can appears flat.</td></tr>
<tr><td colspan="4">Springer - a can with one end permanently bulged. When sufficient pressure is applied to this end, it will flip in, but the other end will flip out.</td></tr>
<tr><td colspan="4">Soft swell - a can bulged at both ends, but not so tightly that the ends cannot be pushed in somewhat with thumb pressure.</td></tr>
<tr><td colspan="4">Hard swell - a can bulged at both ends, and so tightly that no indentation can be made with thumb pressure. A hard swell will generally "buckle" before the can bursts. Burstin g usually occurs at the double seam over the side seam lap, or in the middle of the side seam.</td></tr>
</table>

The number of cans examined bacteriologically should be large enough to give reliable results. When the cause of spoilage is clear-cut, culturing 4-6 cans may be adequate, but in some cases it may be necessary to culture 10-50 cans before the cause of spoilage can be determined. On special occasions these procedures may not yield all the required information, and additional tests must be devised to collect the necessary data. Unspoiled cans may be examined bacteriologically to determine the presence of viable but dormant organisms. The procedure is the same as that used for spoiled foods except that the number of cans examined and the quantity of material subcultured must be increased.

OBSERVATIONS

Particular	Result
Name of Sample	
Exterior Can Condition	
Internal Can Condition	
Micro-leak test	
Product Order	
Product liquor	
Solid Product	
Liquid Product	
Pigment	
Consistency	
Others	

CONCLUSION:

Detection of Bacterial Spoilage of Canned Food

INTRODUCTION:

Generally food is spoiled due to four important reasons. During packaging i) the poor knowledge to the packer ii) mishandling the raw material before packing resulting high level of contamination iii) failure of proper equipment functioning and iv) defect in containers spoiling the canned food. While opening such food containers, a bad smell and acidic taste indicate the food spoilage. Such spoiled food imparts acid and gas production. Sometimes, the tin or packaged item may start swelling due to gas (CO_2, H_2 but not H_2S) formation by anaerobic thermophilic microorganisms. In many cases, the food contents become blackened due to H_2S reaction with iron content of the container material. Common food spoilage occurs due to both aerobic and anaerobic endospore formers of bacilli and clostridia as well as non-spore forming bacteria.

Quantitative analysis of milk by SPC Method

Canned food material to be tested, Gram's stain, Endospore stain, microscope, etc.

MATERIALS REQUIRED:

Materials: Milk sample, Nutrient agar, distilled water

Glasswares and equipments: petri plates, pipettes, incubator, colony counter, etc.

PROCEDURE:

1) Collect such food items from any shop, home, etc and bring it to the laboratory.
2) Transfer the small amount in pre-sterilized beaker through the openings.
3) Prepare Gram-stained and endospore-stained slides from the sample (liquid/semisolid) following the steps.
4) Examine these slides in a bright filed oil immersion

The presence of spore and cells of Gram – stained bacteria indicates the bacteriological food spoilage. Canned food can also made spoiled by irtificial inolcution with *Clostridum thermosaccharolyticum, Clostridium sporogenes, Bacillus coagulans, Bacillus stearothermophillus* or *E. coli.*

OBSERVATIONS:

CALCULATIONS:

Determination of Thermal Death Point (TDP) of microorganism

INTRODUCTION:

Thermal Death Point (TDP) is the temperature at which an organisms is killed in 10 min and thermal death time is the time required to kill the organisms/ spore at a given temperature. Actually, it is necessary to compare the susceptibility of different organisms to rising temperatures. However, some factors such as pH, moisture, composition of media and age of cells influence the TDP. Enzymatic activities of any organism operate well at their optimum temperature. Increase or decrease in temperature influence the microbial growth and survival leading to death also. The degree of tolerance is measured by exposing the microbe to gradually increasing temperature for a given period. E.g. 10 minutes and determining their survival.

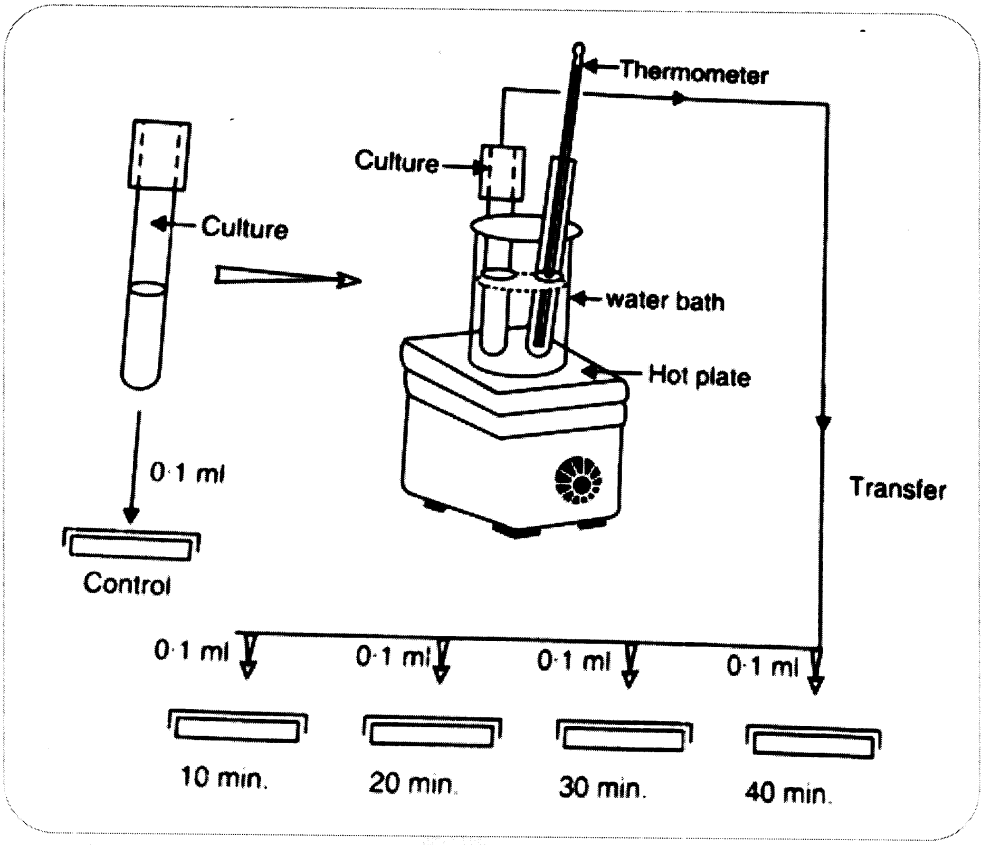
MATERIALS REQUIRED:

Materials: Broth cultures of *E. coli* and *Bacillus cereus*, Nutrient broth tubes

Glasswares and equipments: Thermometer, Glass market, Pipettes, Spreader, Water bath, etc.

PROCEDURE:

1. In order to determine the thermal endurance, heat the culture and a separate tube containing medium in water bath held constantly at 40°C.
2. Place the thermometer in the broth tube.
3. Record the time when the temperature reaches the test temperature.
4. Use the broth cultures *E. coli and Bacillus cereus*.
5. Label 5 plates, first plate as control, second with 10 min, theird with 20 min and fourth with 30 min and fifth with 40 min.
6. Shake the culture and dispense 01. Ml of organisms with a 1 ml pipette to the control plate. Note the time as soon as the temperature of the nutrient browth reaches the desired temperature.
7. After every 10 min, transfer 0.1 ml culture to the plates already labeled as 10 min, 20 min, 30 min, 40 min as shown in figure.
8. Thereafter, pour the liquefied nutrient agar into each plate, rotate the plate for uniform spreading and keep on incubation for 24 to 48 hours.
9. Note down the results.



OBSERVATIONS:

Effect of temperature on survival of *E. coli* and *B. cereus*

Microorganism	Control (°C)	Temperature regime (°C)				TDP
		40	50	60	80	
E. Coli		---				---
B. cereus						
Any other Bacteria						

N.B.: Highest mortality (% dead cells) will be corresponded with that of incubation time, hence maximum TDP will be recorded at 40 minutes in *E. coli* and *B. cereus* may show spore formation at this temperature.

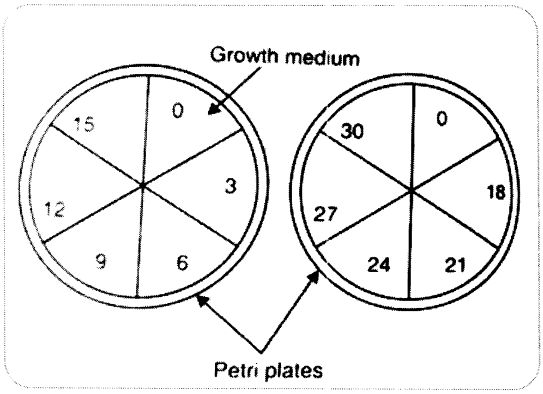
CONCLUSION:

PRACTICAL NO. 12

Determination of Thermal Death Time (TDT) of microorganism

INTRODUCTION:

Thermal Death Time (TDT) is the "time required to kill the cells / conidia/ propagules of an organisms at a given temperature". The TDT differs organisms to organisms. The degree of heat tolerance by microorganisms is determined first by exposing its cells to gradually increasing time, and then assessing its survival by sub-culturing on respective nutriment medium.



MATERIALS REQUIRED:

- Nutrient broth culture of a bacterium (e.g. E. *coli*, *Bacillus subtilis*, etc.)
- Nutrient Agar Medium
- Nutrient Broth Medium
- Water bath
- Thermometer
- Petri Dishes
- Inoculation loop

PROCEDURE:

1. Prepare nutrient broth, pour 2 ml into 11 small sized tubes, ploug properly and autoclave at 121°C for 20 minutes.
2. When cool down, inoculate each tube with a loopful suspension of the bacterial culture and incubate at 30°C for 24 hours.
3. Similarly, prepare nutrient agar medium and pour sterilized and cool down medium into two petri dishes.
4. Take nutrient agar plates and divide into 6 sectors on the back of lower half with the help of a glass marker (pen or Pencil).
5. Label the sector of one plate with intervals to be treated i.e 0, 3, 6, 9, 12 and 15 and other with 0, 18, 21, 24, 27, and 30 minutes. The "0" refers to 0 time or control.
6. Inoculate the 0 sector with suspension of the test bacterium.
7. Note the TDP of the test bacterium.
8. Prepare a water bath and maintain the temperature at 60°C.
9. Put 10 tubes containing 24 hours old bacterial culture into the water bath maintained at 60oC. Now note the time and be careful for sampling.

10. After an exposure of 3 minutes, take out one tube and quickly cool under running tap wate4r.
11. Make streak inoculation of the suspension on sector 3 of the plate.
12. Repeat the process (10 – 11) at every 3 minutes interval i.e. exposure of 6, 9, 12 15, 18, 24, 27 and 30 minutes and make streak inoculation on sector 6 ..... 30, respectively.
13. Incubate the inoculated plates at 30oC for 24 to 48 hours.
14. Tabulate your results.

N.B.: Number 0 – 30 represent the time of exposure ot temperature at 60°C.

OBSERVATIONS:

Particulars	TDT
E. coli	
B. cereus	
Any Other Microbe	

CONCLUSION:

Detection of Salmonella

INTRODUCTION:

Salmonella include gram negative bacilli that are usually found in poultry, eggs, unprocessed milk, meat and water. Members of genus *Salmonella* are motile and possesstwo antigens that play an important role in the identification of these bacteria. These antigens are flaggelar H antigen and somatic O antigen.

MATERIALS REQUIRED:

Selenite cystine (SC) broth, tetrathionate (TT) broth, Rappaport – Vassilidis (RV), bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, hektoin enteric (HE) agar, triple sugar iron (TSI) agar, lysine iron (LI) agar, urea broth, physiological saline, spicer – Edwards flaggelar (H) antisera, phenol red dulcitol broth, trypton broth, polyvalent somatic (O) antisera, MR – VP broth, koser citrate broth, potassium hydroxide, and - naphthol.

PROCEDURE:

Plate count:

- Prepare sample by dissolving 25g of food product in the enrichment broth to have 1:9 ratio, homogenize the food sample and incubate at 35°C for 24hrs.
- Shake the sample after incubation.
- If S. typhi is suspected, Add 1ml of prepared sample to SC broth (10ml) and TT broth (10ml).
- For all other foods, add 0.1ml of sample to RV medium (10ml) and 1ml to TT broth (10ml).
- For foods with high microbial load, Incubate the tubes of RV medium at 42°C for 24hrs. and tubes of TT broth at 43°C.
- For foods with low microbial load, incubate RV tubes at 42°C for 24hrs. and TT at 35°C for 24hrs. for guar gum and foods with S. typhi, incubate the SC and TT tubes at 35°C for 24hrs.
- Vortex the tubes and streak 10l of the culture from all the tubes on BS agar, XLD agar and HE agar. Incubate the plates at 35°C for 24hrs.
- Examine the plates for colonies of Salmonella i.e. yellow colonies with or without black centers.

OBSERVATIONS:

Particulars	Result
Sample – 1	Present / Absent
Sample – 2	Present / Absent
Sample – 3	Present / Absent

CONCLUSION:

Detection Escherichia coli and Coliform

INTRODUCTION:

E.coli is a member of family *Enterobacteriaceae*that also include other bacterial general like *salmonella, shigella* etc. pathogenic strain of *E.clio* include *enteropathogenic E.coli*(EPEC), enterotoxigenic *E.coli* (ETEC), enterohemorrhagic *E. coli* (EHEC) enteroinvasive *E.coli* (EIEC) etc.

E.coli has also found its use as indicator of fecal contamination of food and water. It is found in human and animal gastro-intestinal tract and easily identified due to its capacity to ferment lactose. Based on phenotypic characteristics, they are classified as 'coliforms', which include gram negative, facultative, anaerobic rod shaped bacteria that ferment lactose to produce acid and gas within 48 hrs at 35°C. Their detection is carried out by lactose fermentation test at temperature of around 45°C. the test for coliforms and *E.coli* are based on same principle but have several variation depending upon type of food in which detection has to be carried out.

REQUIREMENTS:

Butterfields phosphate-buffered water, laury sulphate tryptose(LST) broth, brilliant green lactose bile(BGLB) broth,EC broth, Levine-eosin methylene blue agar, (L-EMB), tryptone agar,MR-VP broth,4-methylumbelliferyl βvD–glosuronide(MUG) , M-endo medium,LES endo agar, universal pre enrichment agar, etc.

METHODS OF DETECTION:

Most probable number method (MPN):

Presumptive test for detection of *coliforms*, fecal *coliforms* and *E.coli.*

- Weigh 50 g of sample in a sterile jar add 450 ml of butterfields phosphate –buffered water, blend it for 2 min. This gives a 10^{-1} dilution
- Prepare decimal dilution from first dilution
- Mix the dilution by shaking the bottles
- Transfer 1 ml of dilution to 3 tubes of LST broth each for 3 consecutive dilutions. Transfer 1 ml dilution to 5 LST tubes if analyzing shellfish.
- Incubate all tubes at 35°C for 48 hr and look for gas production after 24 hr and 48 hr of incubation.
- Use tubes showing positive reaction for gas production for MPN confirmed tests.

Confirmation test for coliforms:

- Streak a loopful of culture from EC tube on L-EMB agar and incubate the plate at 35°C for 24 h
- Observe for dark centered flat colonies with green metallic sheen. Only gram-negative bacteria grow on EMB agar. Based on its rate of lactose fermentation ,E. coli produces dark blue –black colonies with a metallic green sheen on EMB agar.

- Transfer the suspected colonies the plate count agar slants and incubate at 35°C for 24 hr
- Use the growth obtained on PCA for further test such as gram staining
- Select gram negative and bacilli shape cultures for further testing .confirm the reaction in LST tube from the PCA culture.

Indole test:

This is positive for bacteria producing tryptophase that leads to formation of indole, inoculate tryptone broth from suspected colonies and incubate at 35°C for 24 hr. add 0.3 ml of Kovac's reagent and consider the appearance of red in upper layer is positive.

Methyl red test:

This test determines the capability of bacterial culture to utilize glucose and produce acidic and products. Inoculate MR-VP broth with suspected culture and incubate at 35°C for 48hr. Add a few drop of methyl red indicator to this culture. Consider the appearance of red color as positive reaction.

Voges-proskauer (VP test):

This test determines the capability of bacterial culture to produce neutral end products by fermenting glucose. Inoculate MR-VP broth with suspected culture and incubate at 35°C for 48 hr. add 0.6 ml α napthol solution and 0.2 ml 40 % KOH. Shake the tub vigorously and then add few crystals of creatine. Shake and let stand for 2 h. consider the development of deep rose color as positive VP test

Citrate test:

This is used to detect citrate utilization by bacterial culture. Kosr's citrate broth and incubate at 35°C for 96 h. consider the development of turbidity as positive reaction for citrate utilization.

Interpretation:

- All the negative rods that ferment lactose with gas production and give typical IMNiC reaction as ++ --and -+-- are categorized as E. coli biotype 1 and biotype 2, respectively.

LST-MUG method for E.coli detection :

This method is for detection of E.coli based on detection of enzyme β glucuronidase (GUD) that cleaves the substrate 4-methylumbilliferyl β–D-glucuronide(MUG) to release 4–methyllumbelliferone (MU). On exposure to 365 nm UV, MU produces bluish fluorescence. This substrate can be added to media and florescence due to production of GUD. However some strains do not produce it.

Procedure:

- Inoculate LST-MUG broth as done in the MPN-presumptive test.
- Incubate it at 35°C for 48 hrs.
- Observe for bluish florescence under UV exposure . compare with the proper negative and positive controls.
- Streak L-EMB plates from the tubes giving positive reaction and continue with the confirmed and complete test as in the above section.

Analysis of bottled water by membrane filter method for coliforms :

- Filter 100 ml of water sample.
- Place the filter surface of M-endo medium
- Incubate the plate at 35°C for 24 hr.
- Observe for the presence of typical colonies that are pink to dark red with a green metallic surface sheen
- Count the typical colonies. If they are in range of 5 -10 in numbers inoculate all in LST medium and incubate at 35°C for 48 hr. if colony numbers is more than 10 randomly pick the exhibiting the typical morphology.
- Look for gas production in LST tubes and subculture from the tubes that are gas positive to BGLB.
- Incubate the tube at 35°C for 48 hr and again observe for gas production.
- Gas evolution in BGLB gives a complete test. Report the result as number of coliforms colonies per 100 ml of sample.

Analysis of E.coli in citrus juices :

- Detection of E.coli in juices cannot be done by standard method s because the pH of these juices is acidic and interferes with the standard tests. The method for detection of E.coli in citrus juices is based on the same principle of lactose fermentation, glucuroidase.etc however it involve as an additional step of pre-enrichment.

Procedure:

- Add 10 ml juice to 90 ml of UPEB and incubate at 35°C for 24 hr
- Transfer 1 ml of enriched sample to 9 ml of EC broth containing coli complete (CC) disc. incubate these tubes at 44°C for 24hr.
- Observe the tubes in long wave length UV light. Appearance of bluish fluorescence indicates the presence of E.coli. appearance of blue color on the surface of discs also indicate the presence of coliforms.

OBSERVATIONS:

Name of Sample	Bottled water
Detection	Present / Absent
Tests	
Indole Test	
Methyl Red Test	
Voges-proskauer (VP test) :	
Citrate Test	

	Result
Name of Sample	Citrus Juice
Detection	Present / Absent
Tests	
Indole Test	
Methyl Red Test	
Voges-proskauer (VP test) :	
Citrate Test	

CONCLUSION:

Detection of Staphylococcus aureus

INTRODUCTION:

S. aureus is spherical shaped, facultative anaerobe, non – spore forming coagulase positive, Gram positive bacteria. It is carried in nasophayrnx region of 50 – 75% of healthy individuals. It is a common cause of mastitis in dairy cattle and hence found in raw milk and dairy products. These bacteria are readily inactivated by heat and sanitizer treatment.

METHODS OF DETECTION:

Baird Parker medium, trypticase soy agar, brain Heart Infusion Broth (BHI) broth, carbohydrate utilization medium, Rabbit plasma with EDTA, Toluidine Blue DNA agar, 0.02M phosphate saline buffer with 1% NaCl, butterfield's phosphate buffered dilution water, sterile paraffin oil, lysostyphin and hydrogen peroxide.

Confirmation test for coliforms:

Streak a loopful of culture from EC tube on L-EMB agar and incubate the plate at 35°C for 24 h Observe for dark centered flat colonies with green metallic sheen. Only gram-negative bacteria grow on EMB agar. Based on its rate of lactose fermentation ,E. coli produces dark blue –black colonies with a metallic green sheen on EMB agar.

PROCEDURE:

Direct plate count method:

- Weigh 50g of food sample in a sterile jar and add 450ml butterfield's phosphate buffered dilution water to it. Blend it at high speed to get 10-1 dilution.
- Prepare the decimal dilutions by adding 10ml of previous dilution to 90ml of sterile diluent.
- Transfer 1ml of each dilution aseptically on three plates of Baird Parker agar, distribute 1ml of inoculum on three plates. Spread the inoculum evenly on the surface of the medium by sterile glass spreader.
- Incubate these plates at 35°C for 45 – 48 hrs. in inverted position.
  Consider the plates having typical *S. aureus* colonies (circular, smooth, convex, moist, gray to jet black).
- Plates having typical *S. aureus* colonies and counting in the range of 20 – 200 colonies are used for determining cfu. However when typical colonies are present at lower dilutions only, then plates with > 200 colonies may be used. In this case, colonies showing typical morphology of *S. aureus* are counted for cfu. Select >1 colony of each counted colony type and test for coagulase production.

Count all the colonies of particular types that give positive coagulase test. Add all such colonies obtained on triplicate plates and multiply with sample dilution factor to report cfu of *S. aureus* per gram of the sample.

Coagulase test:

- Transfer the suspect colonies to narrow tubes containing 0.3ml of BHI broth and emulsify. Inoculate TSA medium with a loopful of BHI suspension and incubate them. (BHI tubes and TSA slants) at 35°C for 24 hrs.
- Add 0.5ml of reconstituted plasma with EDTA to BHI suspension. And incubate at 35°C for 6hrs.
- Examine periodically for clot formation during the incubation period.
- Consider the tubes exhibiting the firm clot as positive for *S. aureus*.
- Keep the positive and negative controls for coagulase test.
- Carry out Gram staining for all the suspect cultures and observe microscopically for presence of gram positive cocci in benches.

OBSERVATIONS:

Particulars	Results
Detection	
Total Plate Count	
Coagulase Test	

CONCLUSION: