MICROBIAL ANALYSIS OF ACTIVE PHARMACEUTICAL INGREDIENT

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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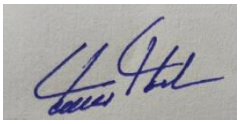
2020-21

ANNEXURE 1

C E R T I F I C A T E

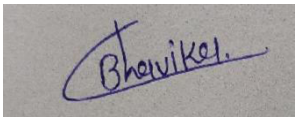
This is to certify that this training report entitled **"Microbial Analysis of Active Pharmaceutical Ingredient"** was successfully carried out by Miss **Suchak Priti Dilipbhai** towards the partial fulfillment of requirements for the degree of Master of Science in Microbiology of Shree M & N Virani Science College, (autonomous),Affiliated to Sauratsra University, Rajkot. It is an authentic record of her own work, carried out by him/her under the guidance of Ms**. Bhavika Chandrani** for a period of **1st February 2021 to 15th April 2021** during the academic year of **2020-2021**. 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.

Mr. Chirag Patel



Name & Signature of the Head of the Department

Ms. Bhavika Chandrani



Name & Signature of the supervisor

ANNEXURE 2

D E C L A R A T I O N

I hereby declare that the work incorporated in the present internship report entitled **" *Microbial analysis of active pharmaceutical ingredient* "** 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.

04 / 05 / 2021. Priti Dilipbhai Suchak

Date (Name and signature of Student)

A C 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 Tyafvallabh 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 .*

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LIST OF TABLES

L I S T O F F I G U R E S

cfu	Colony forming unit
MLT	Microbial limit test
QA	Quality Assurance
QC	Quality control

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

A B S T R A C T

Active pharmaceutical ingredient is any substance or combination of substance user in a finished pharmaceutical product(FPP) , intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, treatment or prevention of disease or to have direct effect in resorting, correcting or modifying physiological functions in human beings. API testing performed in this study is to analyze its activity and capability of ingredient to limit microorganisms. This study was conducted to estimate the presence of microbial contaminants used for the preparation of different pharmaceutical product. A total of 5 samples of API were subjected to total viable count and test for existance of pathogens using standard conventional techniques. Microbial load varied among the APIs. In general bacterial contamination was not more than 1000 cfu/plate and fungal contamination was not more than 100 cfu/ plate in the sample tested. The microbial quality of the tested samples was in general satisfactory. It is by amd large desirable to have information regarding microbial content of all pharmaceutical products,whether sterile or non – sterile consideration must also be given to their proper handling and storage.

I N T R O D U C T I O N

1.0 History

Orchev Pharma Pvt. Ltd. established in 1989 as a single site operation ,Orchev has a reputation for manufacturing Ranitidine as per the latest USP/EP/BP/IP.The entire mission was spearheaded by its visionary leader Mr. Vasant Bhalodiya,who is in the field of chemical engineering since more than 36 years and having in – depth technical expertise of almost all segments of the industry like identifying the product, setting up of the unit ,production,attaining techniqal expertise , developing R & D ,establishing of price structure and also marketing and export. In 2020,orchev has taken next step by introducing few more APIs which are Famotidine, Levetiracetam and Allopurinol.The focus of the company remains to provide high- quality APIs to both regulated and non-regulated markets, which helps to offer their products at economical rates.

There are various departments are present in the company :

1. QA/QC Department
2. Microbiology Department
3. Store & Production Department
4. PA Department
5. Engineering Department
6. Effluent Treatment Plant

1.1 QC Department :-

Quality control is a system of routine technical activities, to measure and control the quality of the invertort as it is being developed. The QC system is designed to : .

- Provide routine and consistent checks to ensure data integrity; correctness and completeness.
- Identify and address errors and omissions.
- Document and archive inventory material and record all QC activites..

It includes :-

1. QC Chemistry department
2. QC Microbiology department
    - Change room
    - Media storage & Media Preparation room
    - Testing room
    - Washing & Cleaning room.

1.2 Entry and exit procedure :-

In microbiology section lab must be properly cleaned and sanitized as to avoid unwanted or foreign particles in the microlab. And also person have to follow appropriate procedure for entry and exit like (As per SOP) .As person enters into the lab

- They have to remove their shoes and wear the lab sleepers or shoes cover
- Sanitize hands with disinfectant.
- Wear clean apron and then enter into the testing area.

1.3 Responsibilities on QC microbiology department

- Microbial contaminants can enter a boo manufacturing production system and impact the product outcomes .Microbiologylist Quality Control department must understand, monitor and prevent these impurities. Daily team protocols must be followed to control environmental factors and maintain sterility.
- Microbiology Quality control department are responsible for assuring quality of the product through all day-to-day operations.They assist the biomanufacturing plant by evaluating raw materials, other supplies and the finished ,packaged product.

- They ensure compliance to common Good Manufacturing Practice .They calibrate and maintain microbiology laboratory equipment.compile and analyze data for documentation,prepare related reports,they revise and update SOPs as necessary.

1.4 Analysis procedure followed by microbiology quality control department

- **SOPs training :-**
    1. General SOP
    2. Operation SOP
    3. Method Of Analysis SOP
    4. Preparation SOP
    5. Calibration SOP
    6. Validation SOP
    7. Cleaning SOP
    8. Others SOP

1. **Chemical and microbiological analysis of process water.**
    a. Chemical analysis of process water
        i. pH
        ii. Conductivity
        iii. Total organic carbon
        iv. Total dissolved solids
        v. Total hardness
    b. Microbiological analysis of process water
        i. Total aerobic microbial count (TAMC)
        ii. Total combined yeast and mold count (TYMC)
        iii. Specified pathogen testing .
2. **Microbiological analysis of API**
    a. Microbial limit test (MLT)
    b. Specified pathogen testing .
3. **Environmental monitoring of microbiology section**

    EM is followed by one method : settle plate technique.

    - Areas of microbiology section that are monitored :-
        - Biosafety cabinet

- Testing room
- Media preparation room

4. **Environmental monitoring of powder processing and packaging area :-**
   a. EM is followed by one method : settle plate technique.
      - Areas of powder processing and packaging section that are monitored :-
        - Change room
        - Passage area
        - Milling and shifting room
        - Centrifuge room
        - Packaging room
        - Quarantine room
        - Drying room
        - Reactor room
5. **Growth Promotion Test (GPT) :-**
   - Media prepared and ready to used media are tested before use to check whether they are capable of providing a luxuriant growth towards specific organisms .
6. **Personnel Monitoring :-**
   - Personnel monitoring is done by the two methods :-
   - Finger dab method and
   - Gown swab method
7. **Validation :-**
   - Validation of autoclave
   - Validation of laminar air flow
8. **Calibration :-**
   - Calibration of pH . There are three types of pH are calibrated (4,7,10).
   - Calibration of analytical balance : (limit : 500 mg to 150 gm)
9. **Staining :-**
   - Gram's staining :
   - Lactophenol cotton blue stain

❖ Instruments which are used in the analysis :-

1. **BOD Incubator**

Incubator is a device used to grow and maintain microbiological culture or cell culture. The incubator maintains optimal temp.,humidity and other conditions such as the carbon and

(Fig.1 Incubator)

oxygen content Of the atmosphere inside. A bacteriological incubator is basically a device which helps in carrying out the process of incubation.Mainly there are two variations in incubators : 1) BOD incubator

2) Bacteriological incubator

2. Hot air oven

Hot air oven are electrical devices which use dry heat to sterilize.Things that are sterilized in a hot air oven include :

- Glasswares (petridishes, flasks ,pipettes and test tubes).
- Powders (starch ,zinc oxide and sulfadizine)
- Materials that contain oils
- Metal equipment (scalpels.scissors and blades).

The time & temprature required are 170 degree for 30 min.,160 for 60 min. And 150 for 15min.

(Fig. 2 Hot air oven)

3. Laminar air flow

The purpose of using such workstations in laboratory is to creat particle and bacteria free working environment to carryout specialized work .As these units discharge air towards user, they provide no personal protection but product protection from room contaminants.

(Fig. 3 LAF)

4. **Autoclave.**

Autoclaves provide a physical method for disinfection and sterilization.Autoclave operates at high temp. and pressure in order to kill microorganisms and spores .They are used to decontaminate certain biological waste and sterilize media, instruments and lab ware .

(Fig. 4 Autoclave)

5. **Centrifuge**

A centrifuge is used to separate particles suspended in liquid according to particle size and density , viscocity of the medium and rotor speed .Within a solution ,gravitational force will cause particles of higher density than the solvent to sink and those less dense than the solvent

to float to the top.

(Fig. 5 Centrifuge)

6. **Dynamic pass box**

Dynamic pass box are used for the transfer of material from one area to another in isolation by means of mechanical / electromagnetic door interlocking process that ensures opening of only one door at a time .This process helps in avoiding cross contamination between two areas.

(Fig. 6 Dynamic pass box)

7. pH meter.

A pH meter provides a value as to how acidic or alkaline a liquid is. The basic principle of the pH meter is to measure the concentration of hydrogen ions .

(Fig. 7 pH meter)

8. Water bath

A laboratory water bath is used to heat samples in the lab.some applications include maintaining cell lines or heating flammable chemicals that might combust if exposed to open flame , A water bath generally consist of a heating unit, a stainless steel chamber ,that holds the water and samples.and a control interface.

(Fig.8 water bath)

9. Weighing Balance

It is a instrument which is used to determine the weight or mass of an object .It is available in a wise range of sizes with multiple weighing capacities. They are essential tools in laboratories, commercial lichens and pharmacies.

(Fig. 9 Weighing balance)

10. Refrigerator

Laboratory refrigerators are used to cool samples or specimens for preservation and It's function is to maintain controlled environment of various fluids and substannces.

(Fig.10 Refridgerator)

11.Microscope

A microscope is an instrument that is used to magnify small objects , some microscopes can even be used to observe an object at the cellular level, allowing to see the shape of a cell, its nuclues, mitochondria and other organelles.

(Fig. 11 Microscope)

Review of Literature

Background

In many WHO guidelines the following definition for an active pharmaceutical ingredient (API) (in the singular) is found under the Glossary (for instance it appears three times in the recently published WHO Technical Report Series, No. 961):

"active pharmaceutical ingredient (API)

- Any substance or combination of substances used in a finished pharmaceutical product (FPP), intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or

to have direct effect in restoring, correcting or modifying physiological functions in human beings."

- This definition implies, for example, that commercially available premixes of APIs (such as the popular amoxicillin + clavulanic acid premix) can be regarded as an API, which is not correct. This definition thus may lead to misinterpretation.
- The current draft Guideline on submission of documentation for a multisource (generic)finished pharmaceutical product (FPP): quality part (QAS/10.373/Rev.1), line 1944, reads:
- "For a mixture of an API with an excipient, the blending of the API with the excipient is considered to be the first step in the manufacture of the final product and, therefore, the mixture does not fall under the definition of an API. The only exceptions are in the cases where the API cannot exist on its own. Similarly, for a mixture of APIs, the blending of the APIs is considered to be the first step in the manufacture of the final product. Sites for such manufacturing steps should be included in this section."

2.0 WHAT IS AN API

Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that when used in the production of a drug becomes an active pharmaceutical ingredient of the drug product(Nighat Razvi ,2014). Such substances are intended to furnish pharmacological activity or other direct efffect in the diagnosis,cure,mitigation,treatment or prevention of the disease or to efffect the structure and function of the body (De La Rosa MC,1995) .

APIs are generally manufactured through a variety of processes that include :-

- Chemical synthesis
- Fermentation process
- Recombinant DNA

- Isolation and recovery from natural sources.
- A combination from these processes.

2.1 Components of medication

All drugs are made up of two core components; the API, which is the central ingredient and the excipients,the substance other than the drug that helps deliver the medication to your system.Excipients are chemically inactive substance, such as lactose or mineral oil. For instance,if you have a headache,acetaminophen is the active ingredient, while the liquid in the gel- capsule or a hulk of a pill is the excipients. (Ifeyinwa F,2006) .

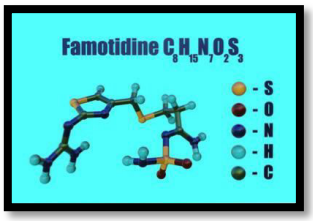
2.2 Strength of an APIs

Manufacturers use certain standards to determine how strong the API is in each drug.However the standard can vary widely from one brand to another. One brand might use one test, another a different one. In all cases, manufacturers are required by the FDA to prove the potency of their product in real life patients, as well as laboratory conditions .

3.0 Different types of APIs

3.1 Famotidine :

Famotidine is a propanimidamide and histamine H2-receptor antagonist with antacid activity. As a competitive inhibitor of histamine H2-receptors located on the basolateral membrane of the parietal cell, famotidine reduces basal and nocturnal gastric acid secretion, resulting in a reduction in gastric volume, acidity, and amount of gastric acid released in response to various stimuli.

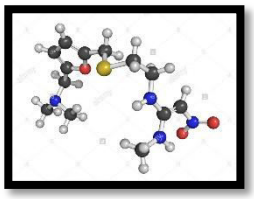


(Fig. 12 Famotidine)

3.2 Ranitidine :

Ranitidine Hydrochloride is a member of the class of histamine H2-receptor antagonists with antacid activity. Ranitidine is a competitive and reversible inhibitor of the action of histamine, released by enterochromaffin-like (ECL) cells, at the histamine H2-receptors on parietal cells in the stomach, thereby

inhibiting the normal and meal-stimulated secretion of stomach acid. In addition, other substances that

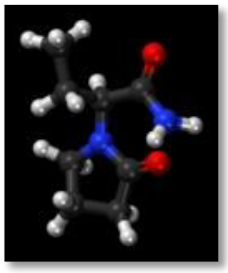


promote acid secretion have a reduced effect on parietal cells when the H2 receptors are blocked.

(Fig.13 Ranitidine)

3.3 Levetiracetam :

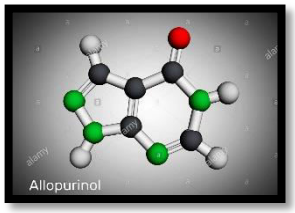
Levetiracetam is a pyrrolidinone and carboxamide that is N-methylpyrrolidin-2-one in which one of the methyl hydrogens is replaced by an aminocarbonyl group, while another is replaced by an ethyl group (the S enantiomer). An anticonvulsant, it is used for the treatment of epilepsy in both human and veterinary medicine.



(Fig. 14 Levetiracetam)

3.4 Allopurinol :

Allopurinol is a structural isomer of hypoxanthine. Allopurinol inhibits xanthine oxidase, an enzyme that converts oxypurines to uric acid. By blocking the production of uric acid, this agent decreases serum and urine concentrations of uric acid, thereby providing protection against uric acid-mediated end organ damage in conditions associated with excessive production of uric acid, i.e. the massive cell lysis

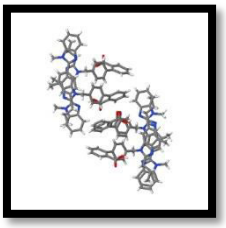


associated with the treatment of some malignancies. (NCI04)

(Fig. 15 Allopurinol)

3.4 Telmisartan :

Telmisartan is a benzimidazole derivative and a non-peptide angiotensin II receptor antagonist with antihypertensive property. Telmisartan selectively antagonizes angiotensin II binding to the AT1 subtype receptor, located in vascular smooth muscle and adrenal gland. The antagonism results in vasodilation and inhibits the angiotensin II-mediated aldosterone production, which in turn leading to a decrease in sodium and water as well as an increase in potassium excretion leading to a subsequent reduction in



blood pressure.

(Fig. 16 Telmisartan)

4.0 General principle of methods :

4.1 Microbial limit test

Microbiological testing plays a significant role in assuring the appropriate quality of drugs. However the paradigm of final product testing particularly for microbiological quality,is shifting because testing alone does not provide complete or absolute assurance for control or absence from microbes (e.g., bacteria , fungi,mycoplasma, viruses) .Additionally ,the reliability of the microbiological testing depends upon the selection of appropriate method that are " Suitable for intended Purpose" and an adequate number of samples taken at appropriate stage of manufacture.For example, to provide an absolute assurance for the absence of microbes in the product, the whole product will be required to be tested for sterility.After the test there will be no product for actual therapeutic use.

4.2 Specified Pathogen Testing

API is a key component of the drug therefore it must be tested for specific pathogen present in it.(Mahboob Hossain,2004) . Pathogens which are liable to be present in API are ,

- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Salmonella abony*

- *Staphylococcus aureus*

4.3 Pour Plate method

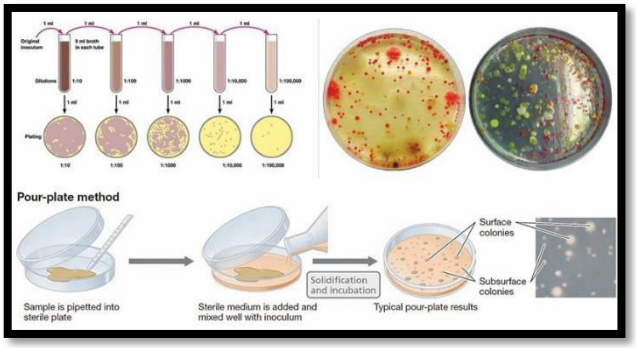
Pour plate technique is a method employed to plate a liquid sample for the purpose of isolating and counting the bacteria present in the that sample.

Active pharmaceutical ingredient is the key component fpr medications therefore it is important to maintain its quality as per the standards.

By performing this study we are able to analyze the ability of API to limit microbial population .

As per the results of bacterial and fungal count it was found to be in a range of <10 cfu/ml .

It shows that the API manufactured complies all the standards and is of excellent quality and can be forwarded for formulation. Pathogen testing showed negative results for all pathogenic organisms. (P.aeruginosa, S.aureus,E.coli and S.abony).



(Fig. 17 Pour plate technique)

5.0 Materials and Methods :

5.1 Material :

5.1.1 Glaaswares and apparatus

1. Sterile test tubes
2. Disposable petriplate
3. Conical flasks
4. Beaker

5.1.2 Equipment / Instrument

1. Hot air oven
2. Horizontal Autoclave
3. Biosafety cabinet / Laminar air Flow
4. Incubator

5. Colony counter

5.1.3 Requirement

1. 70 % isopropyl alcohol

5.1.4 Media

1. Buffer Solution
2. Soyabean casien digest Agar (SCDA) & Soyabean casein digest medium (SCDM)
3. Sabouraud dextrose agar (SDA)
4. MacConkey Agar (MA) & MacConkey Broth (MB)
5. Cetrimide Agar (CA)
6. Mannitol salt Agar (MSA)
7. Xylose lysine deoxycholate agar (XLDA)
8. Rapport Vassiliadis salmonella enrichment broth (RVSEB)

5.2 Microbial analysis of APIs

5.2.1 Pour plate technique

- 10 grams of API sample was added to 100 ml buffer solution.
- 1 ml of sample was pipetted into the 4 empty petriplate.(2 for SCDA & 2 for SDA)

Pouring of molten agar

- Lid of petri plate is slightly lifted and molten SCDA and SDA was poured into petri plates.
- Petriplate were tilted and rotated clockwise to ensure proper mixing of sample and to get uniform layer of agar.
- Petriplates were kept undistributed to solidify.
- Once agar is solidified, SCDA plates were incubated at 32.5± 33.5 °C and SDA plates were incubated at 22.5 °C for 5 days .
- After 5 days of incubation period plates are observed for bacterial colonies and after 7 days fungal colonies were observed.

5.2.2 Specified pathogen testing

- 10 grams of API sample were transferred to 100 ml SCDM.
- SCDM medium were incubated at 30 -35 °C for 24 hours.

- ***Escherichia coli***
    - 1 ml of sample from SCDM was transferred to MacConkey Broth .
    - MacConkey broth was incubated at 42-44°C for 24 hours.
    - After 24 hrs. Incubation, if MacConkey broth show jazzy appreance then proceeded for streaking loopful over MacConkey agar plate.
    - MacConkey agar plates were incubated at 42 – 44° C for 24 hrs.
    - Light pink colour colonies over agar plates shows a positive result .

- ***Salmonella abony***
    - 0.1 ml of sample from SCDM was transferred to RVSEB.
    - RVSEB was incubated at 30 -35 °C for 24 hrs.
    - After 24 hrs. Incubation if RVSEB show hazy appreance then proceed for streaking loopful over XLDA plate.
    - XLDA plates were incubated at 30 -35 °C for 24 hrs.
    - Red colonies with/ without black centers on XLDA plates shows positive results.

- ***Pseudomonas aeruginosa***
    - A loopful from SCDM was streaked on Cetrimide agar plate.
    - Cetramide agar plate were incubated at 30 -35 °C for 24 hrs.
    - Yellowish green colonies on cetramide agar plate shows positive results.

- ***Staphylococcus aureus***
    - A loopful from SCDM was streaked on Mannitol salt agar plate.
    - Mannitol salt agar plates were incubated at 30 -35 ° C for 24 hrs.
    - Yellow colonies on MSA Plate followed by showing colour change (Red to Yellow) in MSA plate which indicates the fermentation of mannitol by *S.aureus*. Hence, positive result.

6.0 Result and Discussion

6.1 Result of Microbial limit test

Sr. No.	API Sample	Bacterial count (100 cfu /ml)	Fungal count (00 cfu/ ml)
1 .	Ranitidine	00	00
2.	Famotidine	03	00
3.	Levetiracetam	00	00
4.	Allopurinol	04	00
5.	Telmisartan	00	00

(Table 1.0 :- Results of microbial limit test)

All of the 5 APIs are tested for bacterial count and fungal count and the results are in the range of 00 to 04 cfu/ ml and NIL for bacterial and fungal count respectively. These results are in range and as per the standards of pharmacopoeia.

6.2Result of pathogen testing

(limit :- should be absent)

Sr. No.	API Sample	*E.coli*	*P.aeroginosa*	*S. abony*	*S.aureus*
1.	Ranitidine	Absent	Absent	Absent	Absent
2	Famotidine	Absent	Absent	Absent	Absent
3.	Levetiracetam	Absent	Absent	Absent	Absent
4.	Allopurinol	Absent	Absent	Absent	Absent
5.	Telmisartan	Absent	Absent	Absent	Absent

(Table 1.1 :- Result of pathogen testing)

Specified pathogen testing for four microorganisms was performed for 5 different APIs, all results were negative for all pathogens.

Conclusion :-

- ✓ pharmaceutical ingredient is the key component fpr medications therefore it is important to maintain its quality as per the standards.
- ✓ By performing this study we are able to analyze the ability of API to limit microbial population .
- ✓ As per the results of bacterial and fungal count it was found to be in a range of <10 cfu/ml .

- ✓ It shows that the API manufactured complies all the standards and is of excellent quality and can be forwarded for formulation. Pathogen testing showed negative results for all pathogenic organisms. (*P.aeruginosa, S.aureus,E.coli* and *S.abony*).

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ANNEXURE :- 1

Preparation of media and reagents

- Soyabean casein digest medium (SCDM)

Ingredients	Gms / Litre
Pancreatic digest of casein	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000

Dextrose	2.500
Dibasic potassium phosphate	2.500
Final pH (at 25°C)	7.3±0.2

- Soyabean casein digest agar (SCDA)

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

- Sabouraud dextrose agar (SDA)

Ingredients	Gms / Litre
Dextrose (Glucose)	40 g
Peptone	10 g
Agar	15 g
Distilled Water	1000 ml
Final pH (at 25°C)	7.3±0.2

- 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

- Cetrimide Agar (CA)

Ingredients	Gms / Litre
Pancreatic Digest of Gelatin	20.0 gm
Potassium Sulfate	10.0 gm
Magnesium chloride	1.4 gm
Cetyltrimethylammonium Bromide	0.3 gm
Glycerine	10.0 ml
Agar	13.6 gm
Distilled water	1000 ml
Final pH (at 25°C)	7.2 +/- 0.2

- Mannitol Salt Agar (MSA)

Ingredients	Gms / Litre
Pancreatic Digest of Casein	5.0 gm
Peptic Digest of Animal Tissue	5.0 gm
Beef extract	1.0 gm
Sodium Chloride	75.0 gm
D – Mannitol	10.0 gm
Phenol Red	0.025 gm
Agar	15.0 gm
Distilled water	1000 ml
Final pH (at 25°C)	7.4 ± 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 phosphate	0.400
Potassium dihydrogen phosphate	0.600
Magnesium chloride,hexahydrate	29.000
Malachite green	0.036
pH after sterilization (at 25°C)	5.2 ± 0.2

ANNEXURE 2

Instrument	Company Name
Water Bath	THERMOLAB
pH meter	EUTECH
Colony Counter	AVM SCIENTIFIC
Microscope	OLYMPUS (CH20i)
Analytical balance	SHIMADZU
Vortex mixture	Eltek
Centrifuge	REMI MOTORS LTD.
Incubators	THERMOLAB
Hot air oven	THERMOLAB
Autoclave	LEQUITRON
LAF	Klenz flo
Refrigerator	VIDEOCON