

INTERNSHIPREPORT
Submitted to
SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

In partial Fulfilment for the Award of The Degree Of

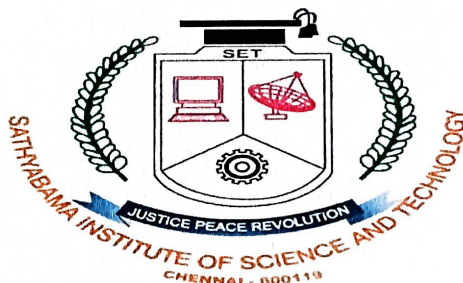
BACHELOR OF SCIENCE

In

MEDICAL LABORATORY TECHNOLOGY

By

Ms.DHIVYA.A(Reg. No: 41737004)



DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY
SCHOOL OF ALLIED HEALTH SCIENCES
SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

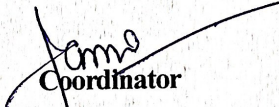
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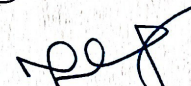
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DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY

BONAFIDE CERTIFICATE

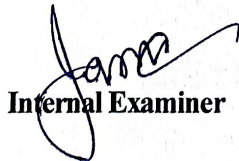
This is to certify that this internship report is the bonafide work of **Ms.DHIVYA.A** who have done the internship work as a team and carried out the training program at “**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY**” and “**EGMORE LIONS BLOOD BANK AND RESEARCH FOUNDATION EGMOR**” under our supervision from August 2023 to April 2024.

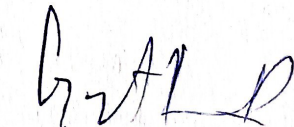

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Submitted for Viva voce examination held on 12th April 2024


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DECLARATION

I, **DHIVYA.A** hereby declare that the internship trainings done by me at **LIONS BLOOD BANK AND RESEARCH FOUNDATION** at **EGMORE** under the guidance of **Ms. D. JASMINE PRIYA** and “**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** at **CHENNAI**” under the guidance of **Dr. JAMES JOHN** at Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of science degree in **MEDICAL LABORATORY TECHNOLOGY**.

DATE: 15.4.2024

PLACE: CHENNAI

A. Dhivya
MS. DHIVYA A

MEDIA PREPARATION

Bacteria and fungi are grown on or in microbiological media of various types. The medium that is used to culture the microorganism depends on the microorganism that one is trying to isolate or identify. Different nutrients may be added to the medium, making it higher in protein or in sugar. Various pH indicators are often added for differentiation of microbes based on their biochemical reactions: the indicators may turn one color when slightly acidic, another color when slightly basic. Other added ingredients may be growth factors, , and pH buffers which keep the medium from straying too far from neutral as the microbes metabolize.

In this exercise, you will make all-purpose media called *trypticase soy broth* and *trypticase soy agar*. These 2 media----one a liquid and the other a solid---are the exact same formula save for the addition of agar agar (really- agar agar), an extract from the cell walls of red algae.

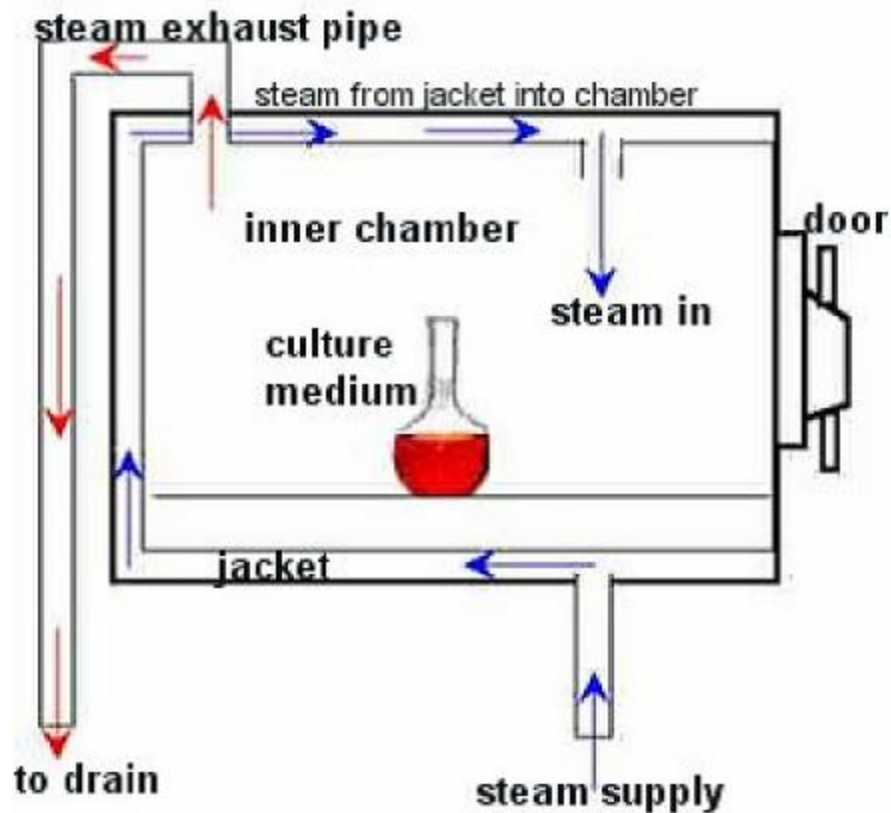
The old way to make media was by the cookbook method--- adding every ingredient bit by bit. The only time that is done today is when making a special medium to grow a certain finicky organism, where particular growth factors, nutrients, vitamins, and so on, have to be added in certain amounts. This medium is called a chemically defined medium (synthetic). Fortunately, the most common bacteria that we want to grow will do nicely with media that we commonly use in lab. Some of our media is bought, but most is produced in the prep area behind the lab. Since this type of medium has some unknown ingredients, or sometimes unknown quantities it is called complex media.

It is really very simple to make complex media these days:

- rehydrate the powder form of the medium
- stir and boil the agar medium to get the agar powder dissolved (if making an agar medium rather than a broth medium)
- distribute the medium into tubes
- autoclave to sterilize the tube media
- autoclave the agar medium for plate production and then pour into sterile petri dishes

STERILIZATION AND THE AUTOCLAVE

When microbiological media has been made, it still has to be sterilized because of microbial contamination from air, glassware, hands, etc. Within a few hours there will be thousands of bacteria reproducing in the media so it has to be sterilized quickly before the microbes start using the nutrients up. The sterilization process is a 100% kill, and guarantees that the medium will stay sterile UNLESS exposed to contaminants by less than adequate aseptic technique to exposure to air.



Media sterilization is carried out with the autoclave, basically a huge steam cooker. Steam enters into a jacket surrounding the chamber. When the pressure from the steam is at a certain point in the jacket, a valve allows the steam to enter the chamber. The pressure will go up over 15 pounds per square inch (psi): at this point the timer begins to count down--- usually for 15 minutes, depending on the type of media. The high pressure in a closed container allows the temperature to go above the highest temperature one could get by just boiling, around 121°C. Therefore, the parameters for sterilization with an autoclave are 121°C at >15 psi for 15 minutes. Fifteen minutes is the thermal death time for most organisms (except some really hardy sporeformers).

The prepared media is distributed in different ways, depending on the form one is making. Broths and agar deeps are dispensed into tubes and then sterilized. Agar slant tubes are sterilized and then the rack is tilted to allow the agar to solidify in a slanted fashion. Agar medium to be poured into plates is sterilized in a flask, and then poured afterward. Not all media or solutions can be sterilized via an autoclave. Certain high-protein solutions such as urea, vaccines, and serum will denature in the extreme heat, and so they may have to be filter-sterilized without heat. You will be making slant and broth media, but not plate media in this lab.

MATERIALS NEEDED (per table)

- 2 plastic weigh boats
- 1 test tube rack
- 1-1 liter Erlenmeyer flask
- 1 pipet pump
- 1 graduated cylinder several nonsterile glass 10 ml pipets
- 1 spatula
- 28 medium, nonsterile test tubes
- 1 jar agar powder 15 green caps
- 1 jar nutrient trypticase soy broth powder
- 15 yellow caps
- 1 magnetic stir bar
- 1 pipet disposal jar

THE PROCEDURE

Refer to the diagram below for the entire production:

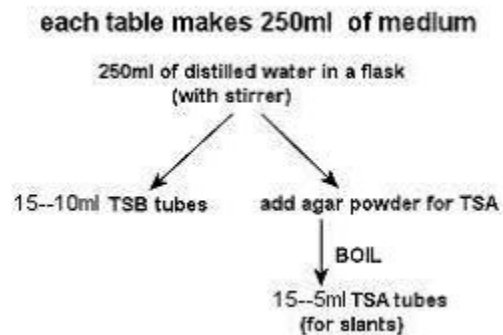


Figure 1: General procedure to make media

1. Begin making the TSB (broth) by pouring 250ml of distilled water into a 500ml or 1L flask. Put in the stir bar and turn on the stir plate so that the surface is just disturbed. Add 3.25 grams of the TSB powder to this flask and allow it to dissolve (will happen quickly). **No heat** need be applied at this stage.
2. Once the powder is dissolved, pipet out 5ml **green** cap.

Green caps are always used for TSB.

3. With the remaining solution (about 100ml) still stirring, add 2 grams of agar powder.
4. The next step will require you to apply heat to the mixture. Before you do this, however, you should be aware that agar has a strong tendency to boil over when it reaches 100°C. Someone in your group should be watching the flask at all times once you see steam coming off of it. At the first sign that the mix is near boiling, **REMOVE** it from the hot plate (paper towels around the flask neck). **DO NOT** simply turn off the heat, letting the flask sit there. The metal plate retains a significant amount of heat, and turning off the heat will not prevent the flask from boiling over. Folded paper towels allow you to grasp the flask neck tightly, yet not burn your hand.
5. Have you read step 4? OK, then you can turn on the heat to setting 9 (not High). Make sure that the magnetic bar is stirring the solution.
6. Upon boiling, the agar dissolves, it will turn clear, deeper tan. Remove it from the heat and pipet out 5ml aliquots into 15 tubes for slants (will not be BE slants until removed from autoclave and tilted to the side to solidify). Cover the slant tubes with yellow caps. **THE REST OF THE AGAR MEDIUM IN THE FLASK WILL BE POURED INTO 1 LARGE FLASK FOR THE CLASS.**

From this point on, yellow caps will be used for nutrient agar slants.

If the agar solidifies in the tip of the pipet, dispose of the pipet in the pipet jar and get another one. To prevent this from happening, either pipet out all the tubes at the same time, or leave the pipet in the flask of melted agar.

7. Place all of the tubes you have pipetted out in the plastic autoclave racks on the instructor's table as well as the remaining of your melted agar. All agar slants go in one rack, broths in another rack, etc.
8. Dispose of your used pipets in the pipet holder. These glass pipets are reusable, so don't throw them in the trash.

Tsi-a

In medical microbiology, TSI (Triple Sugar Iron) agar is a differential medium used to differentiate between different groups of enteric bacteria, primarily *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. TSI agar contains three sugars: glucose, lactose, and sucrose, as well as ferrous sulfate and the pH indicator phenol red.

Here's a basic protocol for preparing TSI agar

Ingredients:

- Peptone
- Beef extract
- Yeast extract
- Sodium chloride
- Lactose
- Sucrose
- Phenol red
- Sodium thiosulfate
- Ferrous sulfate
- Agar
- Distilled water

Glucose

Procedure

- Weigh out the appropriate quantities of each ingredient according to your specific TSI agar recipe.
- Dissolve the peptone, beef extract, yeast extract, sodium chloride, lactose, sucrose, and glucose in distilled water in a large flask or container. Stir to ensure everything is dissolved properly.
- Adjust the pH of the solution to around 7.4 using a pH meter or pH indicator strips.
- Add phenol red to the solution as a pH indicator. Phenol red turns yellow in an acidic environment (due to fermentation of sugars) and remains red in an alkaline environment.
- Add sodium thiosulfate and ferrous sulfate to the solution. These compounds serve as hydrogen sulfide and hydrogen ions indicators, respectively.
- Heat the solution to near-boiling to sterilize it. This can be done either by autoclaving or by heating in a water bath while stirring continuously.
- Once sterilized, cool the solution to around 45-50°C.
- Add agar to the solution as a solidifying agent. The concentration of agar can vary depending on the desired consistency of the agar medium. Typical concentrations range from 1-2%.
- Mix the solution thoroughly to ensure even distribution of agar.
- Pour the agar into sterile petri dishes or tubes, depending on your needs. Allow the agar to solidify at room temperature.
- Once solidified, store the TSI agar plates in a refrigerator at around 4°C until needed.
- Before use, ensure that the TSI agar plates are properly labeled with the necessary information, including date of preparation and contents.
- This is a general protocol, and the specific recipe and procedure may vary depending on the laboratory's protocols and requirements. Always follow the guidelines provided by your institution or laboratory supervisor. Additionally, ensure proper aseptic techniques are used throughout the preparation process to prevent contamination.

Citrate

Preparing citrate media involves creating a culture medium suitable for the growth of microorganisms, particularly those that can utilize citrate as a sole carbon source. Citrate utilization tests are commonly used in microbiology laboratories to differentiate between bacterial species based on their ability to metabolize citrate.

Here's a general procedure for preparing citrate media:

Ingredients:

- Sodium citrate
- Sodium chloride
- Ammonium dihydrogen phosphate
- Dipotassium phosphate

Procedure:

- **Weighing and Measuring:** Weigh out the appropriate amounts of each ingredient according to the desired volume of media to be prepared.
- **Dissolving:** Dissolve the sodium citrate, sodium chloride, ammonium dihydrogen phosphate, dipotassium phosphate, and magnesium sulfate in distilled water. Heating the water can help to dissolve the ingredients more effectively.
- **Adjusting pH:** Adjust the pH of the solution to around 7.0 using a pH meter or pH indicator paper. Citrate media typically has a slightly alkaline pH.
- **Optional Agar Addition:** If making solid media, add agar to the solution. The concentration of agar will depend on whether you want to prepare a solid or semi-solid medium. Typically, agar concentration is around 1.5-2%.

Sterilization: Sterilize the media by autoclaving at 121°C for 15-20 minutes. Ensure proper sealing of containers to prevent contamination

- **Citrate Utilization Test Procedure:**

To perform a citrate utilization test, streak the organism to be tested onto the surface of the citrate agar plate and incubate at the appropriate temperature (usually 37°C for most bacteria). After incubation, observe the plate for growth and color change. Positive citrate utilization is indicated by growth and a change in color of the pH indicator (from green to blue), indicating alkalization due to citrate metabolism.

Always refer to specific laboratory protocols and guidelines for precise measurements and procedures, as they may vary depending on the specific requirements of the experiment and the microorganisms being studied.

MACCONKEY AGAR

MacConkey agar is a selective and differential culture medium primarily used for the isolation and differentiation of Gram-negative bacteria, particularly members of the Enterobacteriaceae family. Here's a basic recipe for preparing MacConkey agar:

Ingredients

- Peptone: 17.0 g
- Proteose peptone: 3.0G
- Lactose: 10.0 g
- Bile salts No. 3: 1.5 g
- Sodium chloride: 5.0 g
- Neutral red: 0.03 g
- Crystal violet: 0.001 g
- Agar: 13.5 g
- Distilled water: 1000 mL

Instructions:

- Dissolve the peptone, proteose peptone, lactose, bile salts No. 3, and sodium chloride in the distilled water by heating and stirring until completely dissolved.
- Adjust the pH to approximately 7.1 using either 1N NaOH or 1N HCl.
- Add the neutral red and crystal violet to the solution. Stir well to ensure even distribution.
- Add agar to the solution and mix well.
- Heat the mixture to dissolve the agar completely.
- Sterilize the medium by autoclaving at 121°C for 15 minutes.
- After sterilization, cool the medium to around 45-50°C.
- Pour the medium into sterile Petri dishes to the desired depth.
- Allow the agar to solidify and then store the plates inverted in a refrigerator at 2-8°C until use.

MacConkey agar is typically a red or pink color due to the presence of neutral red, and it contains lactose as the fermentable carbohydrate. It also contains bile salts and crystal violet, which inhibit the growth of Gram-positive bacteria. Fermenters of lactose such as *E. coli* produce acid during fermentation, causing a drop in pH, resulting in the appearance of pink or red colonies. Non-fermenters such as *Salmonella* and *Shigella* produce colorless colonies.

Blood agar

Blood agar is a type of growth medium used in microbiology to cultivate fastidious organisms, particularly those that require extra nutrients compared to typical bacteria. It's named for the addition of blood, which provides additional nutrients and serves as a differential agent, allowing for the detection of hemolysis (breakdown of red blood cells).

Here's a general protocol for preparing blood agar:

Ingredients

- Tryptic Soy Agar (TSA) base
- Sheep blood (5-10% concentration)
- Distilled water
- Sterile petri dishes or test tubes
- Autoclave or pressure cooker
- Incubator set to appropriate temperature (usually 35-37°C)

Protocol:

- **Prepare Tryptic Soy Agar (TSA) Base:** Follow the manufacturer's instructions to prepare the TSA base. This usually involves dissolving the TSA powder in distilled water, heating to dissolve completely, and autoclaving to sterilize.
- **Add Blood:** Once the TSA base has cooled down to around 45-50°C (to prevent hemolysis), aseptically add the appropriate volume of sterile sheep blood to achieve a final concentration of 5-10%. The exact volume will depend on the manufacturer's instructions and the desired concentration.
- **Mix Thoroughly:** Gently swirl or mix the agar to ensure even distribution of the blood.
- **Pour into Plates or Tubes:** Pour the blood agar mixture into sterile petri dishes or test tubes, being careful to avoid bubbles. Plates should be filled to a depth of around 4-6 mm.
- **Allow to Solidify:** Allow the agar to solidify at room temperature in a level surface or under laminar flow hood.
- **Incubation:** Once solidified, store the plates inverted (agar side up) and incubate them at the appropriate temperature for the growth of your target microorganism. This is typically around 35-37°C for most bacteria.

AGAR AGAR

Agar agar media preparation is a common practice in microbiology laboratories for culturing microorganisms. Agar agar is a gel-like substance derived from seaweed, which acts as a solidifying agent in microbiological media. Here's a general guide on how to prepare agar agar media:

Ingredients

- Agar agar powder
- Distilled water
- Nutrient source (such as peptone, beef extract, yeast extract)
- Additional supplements (depending on the type of media you're preparing)
- pH indicator (optional)

Equipment

- Glassware (flasks, beakers, etc.)
- Stirring rod or magnetic stirrer
- Autoclave or pressure cooker
- pH meter (if using pH indicator)

Steps:

- **Measure Ingredients:** Measure the appropriate amount of agar agar powder and nutrient source according to the recipe or formula you're following. The exact quantities will depend on the specific type of media you're preparing.
- **Mixing:** In a clean container (such as a flask or beaker), mix the agar agar powder and the nutrient source with distilled water. Stir the mixture thoroughly to ensure even distribution.

- **Adjust pH (if necessary):** If your media requires a specific pH, adjust it using an appropriate acid (e.g., hydrochloric acid) or base (e.g., sodium hydroxide). Use a pH meter to ensure accuracy.
- **Autoclaving:** Pour the mixture into sterilizable containers (like culture tubes or petri dishes), leaving some space at the top to prevent overflow during sterilization. Cover the containers loosely with aluminum foil or cotton plugs to allow for steam penetration.

Solmonella shigella

To prepare Salmonella Shigella (SS) agar, you will need the following ingredients and equipment:

Ingredients:

Ingredients:

- **Agar Powder:** Typically available commercially.
- **Peptone:** It's a nitrogen source.
- **Bile Salts:** Used to inhibit the growth of Gram-positive bacteria.
- **Sodium Chloride:** To maintain osmotic balance.
- **Sodium Citrate:** Selectively inhibits the growth of Gram-positive bacteria.
- **Lactose:** Used as a fermentable carbohydrate.
- **Neutral Red:** pH indicator.
- **Brilliant Green:** Inhibits the growth of Gram-positive bacteria.

Preparation Steps:

- **Weighing Ingredients:** Weigh the required amounts of peptone, sodium chloride, bile salts no.3, sodium citrate, lactose, neutral red, and brilliant green. Exact amounts can vary depending on the specific recipe you're following.
- **Mixing:** Dissolve the weighed ingredients in distilled or deionized water in a clean container. Stir gently to ensure even distribution.
- **Adjusting pH:** Measure the pH of the mixture using a pH meter. Adjust the pH if necessary by adding small amounts of acid (such as hydrochloric acid) or base (such as sodium hydroxide) until the desired pH is achieved. For SS agar, the pH typically ranges from 7.2 to 7.6.
- **Adjusting pH:** Measure the pH of the mixture using a pH meter. Adjust the pH if necessary by adding small amounts of acid (such as hydrochloric acid) or base (such as sodium hydroxide) until the desired pH is achieved. For SS agar, the pH typically ranges from 7.2 to 7.6.

- **Adding Agar:** Once the pH is adjusted, add the appropriate amount of agar powder to the mixture while stirring continuously to prevent clumping.
- **Heating and Boiling:** Heat the mixture while stirring until the agar is completely dissolved. Avoid overheating.
- **Autoclaving:** Once all ingredients are dissolved, sterilize the media by autoclaving at 121°C (250°F) for 15 minutes.

XLD

XLD (Xylose Lysine Deoxycholate) agar is a selective growth medium commonly used in microbiology to isolate and differentiate *Salmonella* and *Shigella* species from other Enterobacteriaceae. Here's how you can prepare XLD agar:

Ingredients:

- Peptone: 3 g
- Lactose: 7.5 g

