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Understanding biochemical basis of microbial media

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Abstract

Microbiology has been largely developed thanks to the discovery and optimization of culture media. The first liquid artificial culture medium was created by Louis Pasteur in 1860. Previously, bacterial growth on daily materials such as some foods had been observed. These observations highlighted the importance of the bacteria's natural environment and their nutritional needs in the development of culture media for their isolation. A culture medium is essentially composed of basic elements (water, nutrients), to which must be added different growth factors that will be specific to each bacterium and necessary for their growth. The evolution of bacterial culture through the media used for their culture began with the development of the first solid culture medium by Koch, allowing not only the production of bacterial colonies, but also the possibility of purifying a bacterial clone. The main gelling agent used in solid culture media is agar. However, some limits have been observed in the use of agar because of some extremely oxygen-sensitive bacteria that do not grow on agar media, and other alternatives were proposed and tested. Then, the discovery of antimicrobial agents and their specific targets prompted the emergence of selective media. These inhibiting agents make it possible to eliminate undesirable bacteria from the microbiota and select the bacteria desired. Thanks to a better knowledge of the bacterial environment, it will be possible to develop new culture media and new culture conditions, better adapted to certain fastidious bacteria that are difficult to isolate.

Keywords

Pass Transistor, three-dimensional integrated circuit (3D IC), differential signal transmission.

Introduction to Preparation of Culture Media

The culture media is a mixed nutrient for the growth, reproduction and metabolism of microorganisms. Because microorganisms have different types of nutrition, different requirements for nutrients, and different research purposes, there are many kinds of culture media and different raw materials. But in terms of nutrition, bacterial growthmedia generally contains carbon source, nitrogen source, inorganic salt, auxin, water and so on. In addition, **culture media** has appropriate PH Value, buffer capacity, redox potential and osmotic pressure. In order to meet the specific requirements of research, the preparation and sterilization of culture medium are needed.

Definition of culture media

Culture media, also known as growth media, are specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds). Culture media are used for quality control tests of nonsterile raw materials and finished products as well as for microbial contamination (sterility) tests in applications such as hygiene monitoring, sterilization process validation and determination of the effectiveness of preservatives and antimicrobial agents.

Microbial enumeration tests for nonsterile products are performed according to the harmonized test procedures of the European, US and Japanese pharmacopoeias. These tests, formerly known as Microbial Limit Tests (MLT), determine the bioburden of the product sample. This is achieved by counting the number of colony forming units that have grown on the culture media for a known sample size (CFU/g). Acceptance criteria are based on the Total Aerobic Microbial Count (TAMC), the Total Yeast and Mold Count (TYMC). Furthermore, specific tests are undertaken to confirm the presence or absence of certain microorganisms that may prove to be objectionable.

The culture media ingredients vary according to the test being performed and the microorganism of interest. Most commonly, culture media are based on a nutrient broth (liquid), often mixed with agar and prepared in petri dishes (they may be semi-solid or solid). For enriched or selective media, further specific components are added.

Culture media preparation must be carried out accurately to ensure microbiological growth is correctly promoted. The individual ingredients of the culture media (powders, gels and liquids) must be carefully weighed out according to the culture media formulation recipe. A precision balance with readability from 1 mg up to 10 mg is typically used for the main components. An analytical balance may be required for weighing out trace elements such as copper and zinc. If a smaller amount of culture media is required, care must be taken to correctly recalculate the ingredient quantities and a balance with a higher readability may be required in order to fulfil accuracy requirements.

Microbiological Media Preparation

Overview:

Growth medium or culture medium is a gel or liquid designed to support the growth of microorganisms or cells. There are different types of media for growing different types of organisms or cells. One commonly used type of media is nutrient broth or agar. Some organisms, termed fastidious organisms, require more specialized types of media.

Applicability:

This procedure applies to all University Faculty, Staff, and Students and other university employees who are involved with the preparation of microbiological media.

Storage and Use Requirements:

- Powders to prepare media are stored in general chemical storage.
- Lab coats, gloves and goggles are worn during the preparation of media.
- Use weigh paper or a plastic weigh dish for weighing media powder. Weigh all media in a fume hood.
- Autoclave gloves are worn when handling hot glassware and when using the autoclaves.

General Procedures for Preparing Media Agar Petri Plates:

- Calculate the amount of media that needs to be made.
  - Each plate requires 25 – 30 mL of agar.
  - If 100 plates are needed, 2500-3000mL of agar is needed.
  - Always add 200mL to the amount required in case of spills or miscalculation.

- Follow package instructions for preparation.
  - Instructions are typically written for 1L (1000mL) of media. If less is desired calculate the amount needed as shown:
  - For example: If the instructions state 23g for 1L and 600mL is desired, use a ratio to calculate the amount needed (in this example 13.8 g is needed for preparing 600mL):
  - 23g/1000mL = Xg/600mL
  - 23 * 600= 1000X
  - 13800 = 1000X
  - 13800/1000 =X
  - 13.8 g = X
  - Always prepare media in a beaker with 1/3 of empty space. (i.e. prepare 600mL of media in a 1000mL beaker). If the amount of media to be prepared is greater than 1L, prepare it in 500mL aliquots or use a 2000mL beaker.
  - Label the beaker with autoclave tape and state what media is being prepared, the date, and your initials (i.e. Nutrient Agar 8-18-08 KH).
  - Add powder to beaker first, and then fill with necessary amount of water.
  - Stir with a glass stirring rod to mix. o Place in microwave and heat at 3-5 minute intervals.

- Stir between intervals, using caution and allowing the media to sit for 30 seconds in the microwave before stirring.
- Heat for approximately 10 minutes or until boiling has been achieved.
- Test the pH of the media to insure that it is within the acceptable range as stated on the package. If the pH needs to be adjusted, add drops of 1N Hydrochloric Acid (HCl) (to make more acidic) or 1N Sodium Hydroxide (NaOH) (to make more basic) as necessary until desired pH is achieved.
- Cover the beaker with foil and secure with autoclave tape.
- Autoclave for 20 minutes. Refer to the Standard Operating Procedure for Autoclave Use.
- While the media is in the autoclave, arrange Petri plates on the countertop.
- Once sterilization is complete, open the autoclave and remove the beaker of media. You must wear the autoclave gloves when removing anything from the autoclave.
- Allow media to cool slightly, but not for longer than 10 minutes as the agar may solidify.
- Poke a small hole in the foil covering the top in order to pour the agar.
- Pour approximately 10-15mL of agar into the plates. The bottom of the plate needs to be covered. If necessary, swirl the plate slightly in order to evenly disperse agar.
- Allow plates to cool on the countertop overnight to reduce condensation.
- When cooled, store upside-down in plastic bags in the refrigerator to prevent the agar from drying out.
  - Be sure to seal the plastic bag with masking tape.
  - Be sure the bag is labeled with its contents, the date it was prepared, and your initials (i.e. Nutrient Agar 8-18-08 KH).

Broth Preparation:

- Calculate the amount of media that needs to be made.
  - Each broth tube requires 5-7mL of broth.
  - If 100 tubes are needed, 500-700mL of broth is needed.
  - Always add 200mL to the amount required in case of spills or miscalculation.

- Follow package instructions for preparation.
  - Instructions are typically written for 1L (1000mL) of media. If less is desired calculate the amount needed as shown:
    - For example: If the instructions state 23g for 1L and 600mL is desired, use a ratio to calculate the amount needed (in this example 13.8 g is needed for preparing 600mL):
      - 23g/1000mL = Xg/600mL
      - 23 * 600= 1000X
      - 13800 = 1000X
      - 13800/1000 =X
      - 13.8 g = X
  - Always prepare media in a beaker with 1/3 of empty space. (i.e. prepare 600mL of media in a 1000mL beaker). If the amount of media to be prepared is greater than 1L, prepare it in 500mL aliquots or use a 2000mL beaker.
  - Label the beaker with autoclave tape and state what media is being prepared, the date, and your initials (i.e. Nutrient Broth 8-18-08 KH).
  - Add powder to beaker first, and then fill with necessary amount of water.
  - Stir with a glass stirring rod to mix.
  - Place in microwave and heat at 3-5 minute intervals. o Stir between intervals, using caution and allowing the media to sit for 30 seconds in the microwave before stirring.
  - Heat for approximately 10 minutes or until boiling has been achieved. o While heating, place test tubes in racks and label with autoclave tape (i.e., Nutrient broth 8- 18-08 KH).
  - Test the pH of the media to ensure that it is within the acceptable range as stated on the package. If the pH needs to be adjusted, add drops of 1N Hydrochloric Acid (HCl) (to make more acidic) or 1N Sodium Hydroxide (NaOH) (to make more basic) as necessary until desired pH is achieved.
  - Pour the broth into an appropriately sized glass bottle for pipette dispenser use. o Before attaching the pipette dispenser, set it to the proper setting for the volume of media required for each tube. o Attach the dispenser to the bottle top.
  - While over the sink, test the dispenser to ensure that the liquid media is filling the pipette dispenser. o Fill each tube.
  - Place loose caps on filled tubes.
    - For screw caps, leave the caps partially unscrewed to allow steam to enter and escape.
  - Autoclave for 20 minutes. Refer to the Standard Operating Procedure for Autoclave Use.
  - After sterilization is complete, remove the test tube rack, tighten all test tube caps and allow the tubes to cool at room temperature.

- Once cooled, place in the refrigerator for storage.

Agar Slant Tube Preparation

- Calculate the amount of media that needs to be made.
- Each broth tube requires 7-10mL of broth.
- If 100 tubes are needed, 700-1000mL of broth is needed.
- Always add 200mL to the amount required in case of spills or miscalculation.

- Follow package instructions for preparation.
- Instructions are typically written for 1L (1000mL) of media. If less is desired calculate the amount needed as shown:
  - For example: If the instructions state 23g for 1L and 600mL is desired, use a ratio to calculate the amount needed (in this example 13.8 g is needed for preparing 600mL):
- 23g/1000mL = Xg/600mL
- 23 * 600= 1000X
- 13800 = 1000X
- 13800/1000 =X
- 13.8 g = X
- Always prepare media in a beaker with 1/3 of empty space. (i.e. prepare 600mL of media in a 1000mL beaker). If the amount of media to be prepared is greater than 1L, prepare it in 500mL aliquots or use a 2000mL beaker.
- Label the beaker with autoclave tape and state what media is being prepared, the date, and your initials (i.e. Nutrient Broth 8-18-08 KH).
- Add powder to beaker first, and then fill with necessary amount of water.
- Stir with a glass stirring rod to mix. o Place in microwave and heat at 3–5-minute intervals
- Stir between intervals, using caution and allowing the media to sit for 30 seconds in the microwave before stirring. o Heat for approximately 10 minutes or until boiling has been achieved.
- While heating, place test tubes in racks and label with autoclave tape (i.e., Nutrient Agar Slants 8-18-08 KH).
- Test the pH of the media to ensure that it is within the acceptable range as stated on the package. If the pH needs to be adjusted, add drops of 1N Hydrochloric Acid (HCl) (to make more acidic) or 1N Sodium Hydroxide (NaOH) (to make more basic) as necessary until desired pH is achieved.
- Pour the broth into an appropriately sized glass bottle for pipette dispenser use. o Before attaching the pipette dispenser, set it to the proper setting for the volume of media required for each tube.
- Attach the dispenser to the bottle top.
- While over the sink, test the dispenser to ensure that the liquid media is filling the pipette dispenser.
- Fill each tube. o Place caps on filled tubes.
  - For screw caps, leave the caps partially unscrewed to allow steam to enter and escape.
- Autoclave for 20 minutes. Refer to the Standard Operating Procedure for Autoclave Use.
- After sterilization is complete, remove the test tube rack, tighten all test tube caps, and tilt the tubes of liquid agar on a support that is about ½ inch thick (Plastic weighing dishes work well) or use special white “slant racks”.
- Allow the agar to solidify (about 25 minutes) and store in the refrigerator

First Aid

- In case of contact with eyes, immediately wash the eyes with large amounts of water for 15 minutes, while holding eyelids open. Get medical attention immediately. If contact lenses are worn remove them immediately.
- In the event of skin contact, wash the area thoroughly.
- In the event of skin contact with a hot beaker, seek medical attention.
- Report any incident to the laboratory manager.

Disposal Requirements

- Spills can be cleaned up with a paper towel and disposed of in the trash. Use caution with hot media.
- Unused plates can be disposed of in the waste to be autoclaved trash cans.
- See the microbiological waste SOP for further instruction on waste disposal.

FORMULATION OF CULTURE MEDIADEVELOPMENT AND MANUFACTURE

The formulation of all Oxoid culture media and the components can be divided into different roles or functions:

1. **Nutrients:** proteins/peptides/amino-acids.
2. **Energy**: carbohydrates.
3. **Essential metals and minerals:** calcium, magnesium, iron, trace metals: phosphates, sulphates etc.
4. **Buffering agents:** phosphates, acetates etc.
5. **Indicators for pH change:** phenol red, bromo-cresol purple etc.
6. **Selective agents**: chemicals, antimicrobial agents.
7. **Gelling agent:** usually agar.

There is often an overlap of functions of some media components, thus protein hydrolysates will supply amino-nitrogen, energy, some metals/minerals and act as buffering agents. Phosphate buffers are important suppliers of minerals and agar contributes metals.

1. Nutrients:

Naegeli is credited with the earliest publications (1880/82) describing the requirements of micro-organisms for a protein component which he called `peptone'.

Later work showed that the group of bacteria, now defined as chemo-organotrophs, required amino-nitrogen compounds as essential growth factors in their culture media.

Meat infusions contain water-soluble fractions of protein (amino-acids and small peptides) along with other water-soluble products such as vitamins, trace metals, minerals and carbohydrates (glycogen). Such infusions or extracts may have been regarded as `peptones' but their amino-nitrogen content was usually too low to sustain the growth of large numbers of bacteria.

It was not until deliberate attempts were made to hydrolyse proteins with acids or enzymes that sufficiently high concentrations of water-soluble protein fractions (peptides) were made available for bacterial growth. Many nutrient media usually contain a mixture of protein hydrolysate (peptone) and meat infusion (meat extract/Lab-Lemco).

The difficulties associated with the production of protein hydrolysates were soon recognised and commercial suppliers of peptones became established by the 1920s. The commercial supply of dried peptone eventually led to complete culture media being produced in the form of dehydrated media.

Although meat was the first and most obvious protein to hydrolyse, other proteins were tried later and some showed specific advantages which ensured their retention in culture media to this day. Casein hydrolysate with its pale colour and high tryptophan content and soya peptone with its high energy carbohydrate content are popular examples of non-meat peptones.

A detailed description of these products is given in "Peptones-Hydrolysates" section.

The nutrient components of culture media are carefully selected to recover the required spectrum of organisms in the sample e.g. coliforms or anaerobes. General purpose media such as blood agar in its various forms will often contain mixtures of peptones to ensure that peptides of sufficient variety are available for the great majority of organisms likely to be present. However, more demanding organisms will require supplemental growth factors to be added and examples of such requirements can be seen in media for *Legionella* species.

Most of the components used for the nutrition of micro-organisms are undefined and require extensive testing with careful selection to ensure a reasonable degree of uniformity. Would it not be better to use wholly defined peptides and amino-acids to produce a totally defined medium? Whilst such media would improve uniformity, experience has shown that they lack good performance as general purpose media. They would also be very expensive compared with undefined media. The use of totally defined culture media is an understandable goal of most microbiologists but defined media have yet to prove themselves equal in performance to currently used complex mixtures of meat and plant protein hydrolysates.

2. Energy

The most common substance added to culture media as a source of energy to increase the rate of growth of organisms is glucose. Other carbohydrates may be used as required.

Carbohydrates added to media at 5-10 grammes per litre are usually present as biochemical substrates to detect the production of specific enzymes in the identification of organisms. It is usual to add pH indicators to such formulations.

3. Essential Metals and Minerals

The inorganic essential components of culture media are many and can be divided on a semi-quantitative basis:
Typical macro-components (gm/litre): Na, K, Cl, P, S, Ca, Mg, Fe.
Typical micro-components (mgm-microgm/litre): Zn, Mn, Br, B, Cu, Co, Mo, V, Sr, etc.
As previously mentioned, a formulation may not have specific metals and minerals listed in its formulation. In such cases it is assumed that all the factors required are present in the hydrolysates, buffers and agar components.

4. Buffering Agents

It is important that the pH of a culture medium is poised around the optimum necessary for growth of the desired micro-organisms. The use of buffer compounds at specific pK values is especially necessary when fermentable carbohydrates are added as energy sources.

Phosphates, acetates, citrates, zwitterion compounds and specific amino-acids are examples of buffering agents that may be added to culture media.

A side effect of such compounds is their ability to chelate (or bind) divalent cations (Ca ++ and Mg ++). Polyphosphate salts, sometimes present in sodium phosphate, are compounds which can bind essential cations so firmly that they are made inaccessible to the micro-organisms.

The effect of these binding or chelating agents will be seen in diminished growth or failure to grow at all, unless care has been taken to supplement the essential cations in the formulation. Opacity forming in a medium, after heating or on standing at 50°C for several hours, is commonly caused by phosphate interaction with metals. Such phosphate precipitates can very effectively bind Fe and lower the available amount of this essential metal in the medium.

5. Indicator Substances

The addition of coloured indicator substances is a very effective way of detecting fermentation of specific carbohydrates in a culture medium. Such compounds should change colour distinctly and rapidly at critical pH values.

Most of the compounds used e.g. phenol red, bromo-cresol purple, fuchsin, etc., are toxic and it is essential to use low concentrations of pre-screened batches/lots. Known sensitive strains of micro-organisms are used in the screening tests.

6. Selective Agents

Chemicals or antimicrobials are added to culture media to make them selective for certain micro-organisms. The selective agents are chosen and added at specific concentrations to suppress the growth of unwanted organisms in a polymicrobial sample. It is, of course, essential to have established that the selective agents, at the appropriate concentration, will allow uninhibited growth of the desired organisms.
Common chemical selective agents are: bile salts, dye-stuffs, selenite, tetrathionate, tellurite and azide. Antimicrobial agents are commonly used in mixtures when suppressing polymicrobial contaminating flora. Antimicrobials are more specific in their selective action than the chemical agents shown above. However, the critical weighing and heat-lability of most antimicrobials demand special care and post-sterilisation addition.
The wide variety of organisms and their almost infinite ability to adapt to changing conditions makes a truly selective medium unlikely. Selective media can be said to suppress most of the unwanted organisms and allow most of the desired organisms to grow. The final formulation is usually a compromise which achieves the best of these criteria.

7. Gelling Agents

Although gelatin is still used for a few specific media and carrageenans, alginates, silica gel and polyacrylamides are sometimes used as gelling agents, the outstanding gel-forming substance used in culture media is agar.

Hesse, a worker in Robert Koch's laboratory, is credited with its first use in culture media, although Frau Hesse gave him the idea from its use in table-jellies in hot climates.

Its inertness to microbial action, the unique setting and melting temperatures (38°C and 84°C respectively) the high gel strength which allows low concentrations of agar to be used, its clarity and low toxicity have contributed to its wide popularity with microbiologists. Its ability to retain its gel structure at 60°C makes agar of special value to culture media which have to be incubated at this temperature to isolate thermophilic organisms.

Agar is obtained from agarophyte sea-weeds mainly *Gelidium, Gracilaria* and *Pterocladia* species. It is extracted as an aqueous solution at greater than 100°C, decolourised, filtered, dried and milled to a powder.

Agar is not an inert gelling agent; it contributes nutrients and/or toxic agents to culture media, depending on the chemical processing carried out by the suppliers.

Microbiological agar is specially processed to yield a low toxicity, high clarity, low mineral and high diffusion gel.

Other Components

There are many other substances added to culture media for specific purposes e.g. growth factors for fastidious organisms, eH-reducing compounds for anaerobic organisms (thioglycollate and cysteine), whole blood to detect haemolytic enzymes and encourage the growth of organisms which are vulnerable to oxidation products.

Types of Culture Media

1. Basal Media
2. Enriched Media
3. Selective
4. Indicator Media
5. Transport Media
6. Storage Media

1. Basal Media

A 40% rumen fluid basal medium has been developed that without added substrxate will support growth of about 10% or less of the total colony count obtained with 40% rumen fluid-glucose-cellobiose-starch-agar medium (RGCSA). The basal medium is prepared by anaerobic incubation of all ingredients in RGCSA medium except the carbohydrates, Na2CO3, and cysteine for 7 days at 38 degrees C. After incubation, substrate(s), Na2CO3 and cysteine are added and the medium is tubed and sterilized as in normal medium preparation. When xylose was included with glucose, cellobiose, and starch as added carbohydrates in the incubated medium, colony counts were comparable to those obtained with RGCSA medium. The addition of specific carbohydrates or other substrates as energy sources to the basal medium suggested that the percentage of the bacterial population capable of utilizing these energy sources was influenced by the ration of the animal; however, considerable animal variation and day-to-day variation in a given animal was observed. Comparison of the population in animals fed either orchardgrass hay or 60% corn-40% orchardgrass (60-40) indicated little or no difference for the percentage of bacteria utilizing glucose, pectin, xylan, or mannitol. Increases in the percentages of xylose-, cellobiose-, Glycerol-, and lactate-utilizing bacteria occurred with the orchardgrass hay ration, whereas the percentage of starch-digesting bacteria was increased significantly (P less than 0.01) in the animals fed the 60-40 ration. A limited number of bacterial strains were isolated from the basal medium without added substrate, most of which were atypical with respect to the predominant rumen bacteria. Growth of these strains, even in complex media, was very slow and limited. Based on these data with isolated strains and colony counts obtained in roll tube medium containing only minerals, resazurin, agar, Na2CO3, and cysteine, the selective medium overestimated the percentage of bacteria able to use a specific energy source. This overestimate was 6 to 7% of the total culturable count.

2. Enrich Media

Enrichment Medium is a highly nutritive medium which can be used as a general purpose enrichment agar base.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	40.000
Yeast extract	6.000
Dipotassium phosphate	3.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 64 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Enriched media contain the nutrients required to support the growth of a wide variety of organisms, including some of the morefastidious ones. They are commonly used to harvest as many different types of microbes as are present in the specimen(1).

Enrichment medium is general purpose enrichment agar which can nourish and support the growth of gram-positive as well asgram-negative bacteria. It can also be supplemented with blood for enriched growth or study the haemolysis.

This medium contains peptic digest of animal tissue and yeast extract which serves as source of nitrogen, carbon, amino acids,vitamins and growth factors for growth of bacteria. Dipotassium phosphate buffers the medium well. Agar is solidifying agent.

Quality Control

Appearance

Light yellow coloured homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gelColour and Clarity of prepared mediumLight amber coloured clear gel forms in Petri plates.

Reaction

Reaction of 6.4% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M318: Cultural characteristics observed after an incubation at 35 - 37°C after 24 hours.

Organism	Inoculum	Growth	Recovery
Cultural Response			
Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Salmonella Typhi ATCC 6539	50-100	luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%

Storage and Shelf Life:

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry date on the label.

3. Selective Media:

Selective Agar Base is used for the isolation and cultivation of Pseudomonas solanacearum.

Composition**

Ingredients	Gms / Litre
Mannitol	2.500
L-Glutamic acid	1.000
Magnesium sulphate, 7H2O	0.160
Manganese sulphate, H2O	0.310mg
Potassium phosphate, monobasic	0.027mg
Zinc sulphate, 7H2O	0.550mg
Ferric ammonium sulphate, 6H2O	0.090mg
Copper sulphate, 5H2O	0.010mg
Calcium sulphate, 5H2O	0.010mg
Phosphoric acid	0.005mg
Potassium iodide	0.000006mg
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters.

Directions

Suspend 18.66 grams in 990 ml distilled water. Mix thoroughly. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of SM Selective Supplement (FD122) and 10 ml of TTC Solution, 1% (FD057). Mix well and pour into sterile Petri plates or as desired.

Principle And Interpretation

Phytopathogenic pseudomonads are a very diverse group of bacteria with respect to their genetics, ecology and the kinds of disease they cause. Some of the worlds most serious bacterial diseases are caused by pseudomonads such as Pseudomonas solanacearum (1).

The bacterium P. solanacearum is the causative agent of bacterial wilt in plants and is the most important and widely spread bacterial diseases of crops in the tropic, subtropics and warm temperate regions of the world (2). SM Selective Agar Base is recommended for isolation and cultivation of P. solanacearum (3).
P. solanacearum is a plant pathogen and utilizes mannitol as carbon source. The various salts added in trace amounts enhance the growth of P. solanacearum , while antibiotic solution serves to inhibit contaminating heterotrophic microflora from samples. The dye 2, 3, 5-triphenyl tetrazolium chloride (TTC) is used as indicator of oxidation-reduction state of the medium. P. solanacearum being highly oxidative, its colony takes up the pink colour of the oxidized dye.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling Firm

Comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates.

Reaction

Reaction of 1.87% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH 6.80-7.20

Cultural Response

M1289: Cultural characteristics observed after an incubation at 30°C for 48 hours with added SM Selective Supplement (FD122) and 10ml of TTC Solution 1% (FD057).

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Pseudomonas aeruginosa ATCC 27853	50-100	none-poor	<=10%
Pseudomonas solanacearum ATCC 11696	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

4. Indicator Media

Composition**

Ingredients	
Acid fuchsin	0.5 gm
1N Sodium hydroxide	16.0ml
Distilled water	984.0ml

Note: Add few more drops of 1N Sodium hydroxide if the reagent is not sufficiently decolourized
**Formula adjusted, standardized to suit performance parameters

Directions

Andrade's Indicator has wide range of application so follow apropriate direction as per application protocol.

Principle And Interpretation

Andrade indicator is a solution of acid fuchsin which when titrated with sodium hydroxide changes color from pink to yellow. It is also used to differentiate microorganisms on the basis of carbohydrate fermentation in broth as well as agar media. Andrade Peptone Water and CLED Agar with Andrade Indicator are commonly used broth and agar media respectively. It becomes pink at acidic pH level (pH 5.0) and yellow at alkaline pH level (pH 8.0).

Type of specimen

Biological sample

Specimen Collection and Handling

Follow appropriate techniques for handling specimens as per established guidelines.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Intended Use:

Andrade's Indicator is recommended to differentiate microorganisms on basis of carbohydrate fermentation.

Limitations

1. An indicator is not functional above its pH range because the indicator does not change color at these pH values.
2. If the substance or sample is contaminated, the color may be wrong.
3. Acid-base indicators show just one- or two-color changes.
4. Indicators measure pH at low accuracy, they only indicate sample acidity or alkalinity and not exact pH

Performance and Evaluation

Performance of the product is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to brownish yellow colored liquid.

Clarity

Clear without any particles.

Reaction

It becomes pink at acidic pH levels and yellow at alkaline pH levels (pH range 5 - 8).

Storage and Shelf Life

Store between 10- 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

5. Transport Media

Intended use

Recommended for the preservation and transportation of Neisseria species and other fastidious organisms from the clinic to laboratory.

Composition**

Ingredients	Gms / Litre
Sodium glycerophosphate	10.000
Sodium thioglycollate	1.000
Calcium chloride	0.100
Methylene blue	0.002
Agar	3.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters.

Directions

Suspend 14.1 grams in 1000 ml double purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes with screw caps to give a depth of approximately 7 cm. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and after sterilization tighten the caps. Cool the tubes immediately in an upright position. Care should be taken that the water is free from chlorine.

Principle And Interpretation

Stuart Transport media were originally designed by Stuart while studying Gonococci (6). Stuart et al (7) later on modified the Stuart Medium for the transportation of gonococcal specimens for culturing. Ringertz included thioglycollate in the Stuart Medium and omitted charcoal (5). The medium may be used for the transportation of many fastidious organisms including anaerobes by maintaining the organism's viability without significant multiplication (4). Crooks and Stuart (1) suggested the addition of Polymyxin B sulphate which facilitates the recovery of Neisseria gonorrhoeae.

This medium is a chemically defined, semisolid, non-nutrient medium which prevent microbial proliferation. Because off this composition the medium ensures that microorganisms present are able to survive for a sufficiently long period of time. The medium provides an adequate degree off anaerobiosis which can be monitored by means off the redox indicator methylene blue. Prepared sterile medium will undergo a slight degree off oxidation at the upper periphery of the medium, however, in the tube or vial exhibits a distinct blue colour throughout the medium, it should be discarded. Calcium chloride along with sodium glycerophosphate act as good buffering agent and also maintains osmotic equilibrium in the medium.

Type of specimen

Clinical samples - Gonococcal specimens.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective cloves/protective clothing/ eye protection/ face protection. Follow good microbiological lab practices

while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

Due to nutritional variations, some strains may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

White to light blue colored homogeneous free flowing powder.

Gelling

Semisolid, comparable with 0.3% Agar gel.

Color and Clarity of prepared medium

Colorless to whitish colored slightly opalescent butt with upper 10% or less portion blue on standing.

Reaction

Reaction of 1.41% w/v aqueous solutions at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 72 hours when subcultured from Stuart Transport Medium.

Organism	Growth	Subculture Medium
Haemophilus influenzae ATCC 49247	good	Chocolate Agar (incubated in CO2 atmosphere)
Neisseria gonorrhoeae ATCC 19424	good	Chocolate Agar (incubated in CO2 atmosphere)
Streptococcus pneumoniae ATCC 6303	good	Tryptone Soya Agar with 5% sheep blood

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 5-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

6. Storage Media

Product Description

Cell Freezing Media are complete, ready to use reagents designed to protect and preserve cells during frozen storage. These media are a convenient and cost-effective alternative to in-house freezing media and can be used for a wide variety of mammalian cells.
Cell Freezing Medium with DMSO is fully supplemented formulation prepared in Dulbecco's Modified Eagle Medium. It contains 10% DMSO and foetal bovine serum. DMSO acts as a cryoprotectant and prevents formation of ice crystals and prevents cell damage. It is ready to use and does not require further addition of any other reagents.

This cryopreservation medium can be used for hardy cell lines that are less susceptible to freezing damage. Users are advised to test the suitability of the medium for sensitive cell lines.

Composition

Ingredients	% (V/V)
OTHERS	
Dimethylsulphoxide (DMSO)	10%
Dulbecco's Modified Eagle Medium	Made to volume
Fetal bovine serum	Proprietary

Directions

Cell freezing medium can be used with standard freezing protocols. The following protocol may be used. Thaw cell culture freezing medium, mix well, and keep on wet ice during use.

Procedure for freezing

1. For optimum results, cells should be in log phase of growth.
2. Gently detach adherent cells from the surface using Trypsin or other appropriate means
3. Gently pellet the cell suspension by centrifugation (200 to 400 x g for 5 minutes for suspension cells and 200 x g for 5 minutes for adherent cells). Using a pipette, remove the medium above the pellet down to the smallest volume without disturbing the cells.
4. Resuspend the cells in Cell Freezing Medium at a recommended density for a specific cell type. Hybridoma cells may require higher cell density.
5. Aliquot cells in appropriate cryogenic storage vials. Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1ºC per minute. Alternatively, place the cryovials containing the cells in an isopropanol chamber and store them at -80ºC overnight. Alternatively, store them at -20ºC for 1 - 2 hours before shifting to -80ºC overnight.
6. Transfer cryovials to liquid nitrogen tank for long term storage.
7.

Procedure for thawing of cryopreserved cells

1. Remove cells from frozen storage and quickly thaw in a 37ºC water bath.
2. Dilute 1ml suspension with 10ml of complete growth medium.
3. Mix cells gently and pellet by gentle centrifugation.
4. Discard the supernatant and gently resuspend the cells in complete growth medium and seed in appropriate culture vessel.
5. It is recommended to do viability assessment 24hours post thawing. For accurate assessment, it is recommended to use fluorescent assay like EZBlue™ assay (CCK004 - EZBlue™ Cell Assay Kit) or metabolic assays like MTT assay (CCK003-EZcount™ MTT Cell Assay Kit).

Notes

1. Cells harvested for cryopreservation should be at their optimum viability to ensure maximum survival during freezing and after thawing.
2. On removal from storage, extreme caution must be exercised to prevent explosion of the cryovial because of sudden expansion of the trapped nitrogen.
3. To retain maximum viability during cryopreservation, cells must be cooled at a constant slow rate, -1 to -5ºC/min. This can be achieved using programmable freezers or placing ampoules in a heavily insulated box at -80ºC for 24 hours before transferring them to their final storage location.
4. After thawing cells, it is necessary to slowly dilute the croprotectant to prevent osmotic shock. When it is necessary to centrifuge the cells, use the minimum g force to sediment them to prevent shearing damage, i.e. 70-100g.
5. To initiate rapid growth, it is advisable to inoculate new cultures at a higher density than for routine subculture, e.g., between 3 and 4 X 104 viable cells/cm2 for adherent cells.
6. The minimum number of tests that should be carried out on master cell banks are, total and viable cell counts, growth potential, screening for bacteria, fungi and mycoplasma and cell line authenticity.

Quality Control

Appearance

Red colored clear solution.

pH

7.60 -8.20

Osmolality in mOsm/Kg H2O

1800.00 -2200.00

Sterility

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Performance Test

Performance test is done by freezing cells and doing a viability assessment after thawing and comparing it with a control medium.

Storage and Shelf Life

Cell Freezing Media should be stored at -20°C. For frequent use, cell freezing medium once thawed can be stored at 2-8°C up to 5 days.The shelf life of product is 12 months. Use before expiry date given on the product label.

Uses of Culture Media

Culture media is the food used to grow and control microbes.

Microbiological Cultures

Culture medium or growth medium is a liquid or gel designed to support the growth of microorganisms. There are different types of media suitable for growing different types of cells. Here, we will discuss microbiological cultures used for growing microbes, such as bacteria or yeast.

NUTRIENT BROTHS AND AGAR PLATES

These are the most common growth media, although specialized media are sometimes required for microorganism and cell culture growth. Some organisms, termed fastidious organisms, need specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells. Many human microbial pathogens also require the use of human cells or cell lysates to grow on a media.

The most common growth media nutrient broths (liquid nutrient medium) or LB medium (Lysogeny Broth) are liquid. These are often mixed with agar and poured into Petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured. They remain solid, as very few bacteria are able to decompose agar. Many microbes can also be grown in liquid cultures comprised of liquid nutrient media without agar.

Microbial pathogen growing on blood-agar plate: Red blood cells are used to make an agar plate. Different pathogens that can use red blood cells to grow are shown on these plates. On the left is staphylococcus and the right streptococcus.

DEFINED VS UNDEFINED MEDIA

This is an important distinction between growth media types. A defined medium will have known quantities of all ingredients. For microorganisms, it provides trace elements and vitamins required by the microbe and especially a defined carbon and nitrogen source. Glucose or glycerol are often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources. An undefined medium has some complex ingredients, such as yeast extract, which consists of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.

There are many different types of media that can be used to grow specific microbes, and even promote certain cellular processes; such as wort, the medium which is the growth media for the yeast that makes beer. Without wort in certain conditions, fermentation cannot occur and the beer will not contain alcohol or be carbonated (bubbly).

COMMON BROADLY-DEFINED CULTURE MEDIA

Nutrient media – A source of amino acids and nitrogen (e.g., beef, yeast extract). This is an undefined medium because the amino acid source contains a variety of compounds with the exact composition being unknown. These media contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory-culture collections.

Minimal media – Media that contains the minimum nutrients possible for colony growth, generally without the presence of amino acids, and are often used by microbiologists and geneticists to grow "wild type" microorganisms. These media can also be used to select for or against the growth of specific microbes. Usually, a fair amount of information must be known about the microbe to determine its minimal media requirements.

Selective media – Used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing.

Differential media – Also known as indicator media, are used to distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection and identification of microorganisms.

These few examples of general media types provide some indication only; there are a myriad of different types of media that can be used to grow and control microbes.

Complex and Synthetic Media

In defined media all the chemical compounds are known, while undefined media has partially unknown chemical constituents. There are many types of culture media, which is food that microbes can live on. Two major sub types of media are complex and synthetic medias, known as undefined and defined media.

Undefined Media: Luria Broth as shown here is made with yeast extract, as yeast extract is not completely chemically defined. Luria Broth is therefore an undefined media. By Lilly_M (GFDL (http://www.gnu.org/copyleft/fdl.html), CC-BY-SA, via Wikimedia Commons

An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.A defined medium (also known as chemically defined medium or synthetic medium) is a medium in which all the chemicals used are known, no yeast, animal, or plant tissue is present. A chemically defined medium is a growth medium suitable for the culture of microbes or animal cells (including human) of which all of the chemical components are known. The term chemically defined medium was defined by Jayme and Smith as a 'Basal formulation which may also be protein-free and is comprised solely of biochemically-defined low molecular weight constituents.

Chemically defined medium is entirely free of animal-derived components (including microbial derived components such as yeast extract) and represents the purest and most consistent cell culture environment. By definition chemically defined media cannot contain either fetal bovine serum, bovine serum albumin, or human serum albumin as these products are derived from bovine or human sources and contain complex mixes of albumins and lipids. The term 'chemically defined media' is often misused in the literature to refer to serum albumin-containing media. Animal serum or albumin is routinely added to culture media as a source of nutrients and other ill-defined factors, despite technical disadvantages to its inclusion and its high cost. Technical disadvantages to using serum include the undefined nature of serum, batch-to-batch

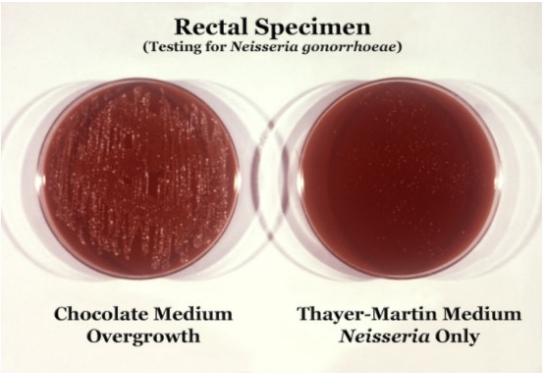
variability in composition, and the risk of contamination. There are increasing concerns about animal suffering inflicted during serum collection that add an ethical imperative to move away from the use of serum wherever possible. Chemically defined media differ from serum-free media in that bovine serum albumin or human serum albumin with either a chemically defined recombinant version (which lacks the albumin associated lipids) or synthetic chemical such as the polymer polyvinyl alcohol which can reproduce some of the functions of serums.

Selective and Differential Media

There are many types of media used in the studies of microbes. Two types of media with similar implying names but very different functions, referred to as selective and differential media, are defined as follows.

Selective media are used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid such as proline in conjunction with E. coli unable to synthesize it were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes. Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite. Normally, the presence of a specific gene or an allele of a gene confers upon the cell the ability to grow in the selective medium. In such cases, the gene is termed a marker. Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as a marker. Gancyclovir is an exception to the rule as it is used to specifically kill cells that carry its respective marker, the Herpes simplex virus thymidine kinase (HSV TK). Some examples of selective media include:

- Eosin methylene blue (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram-negative bacteria.
- YM (yeast and mold) which has a low pH, deterring bacterial growth.
- MacConkey agar for Gram-negative bacteria.
- Hektoen enteric agar (HE) which is selective for Gram-negative bacteria.
- Mannitol salt agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol.
- Terrific Broth (TB) is used with glycerol in cultivating recombinant strains of Escherichia coli.
- Xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, especially Legionella pneumophila.



Non-selective versus selective media.: The non-selective media on the right allows of the growth of several different bactarial species and is overgrown with bacteria (whitish lines). While the plate on the right selectively only allows the bacteria Neisseria gonorrhoeae, to grow (white dots).

Differential media or indicator media distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria. Examples of differential media include:

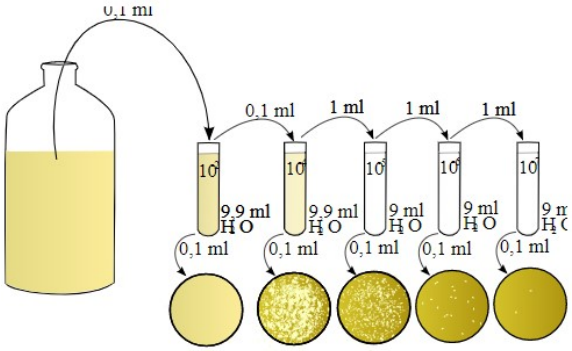
- Blood agar (used in strep tests), which contains bovine heart blood that becomes transparent in the presence of hemolytic.
- Streptococcus eosin methylene blue (EMB), which is differential for lactose and sucrose fermentation.
- MacConkey (MCK), which is differential for lactose fermentation mannitol salt agar (MSA), which is differential for mannitol fermentation.
- X-gal plates, which are differential for lac operon mutants.

Aseptic Technique, Dilution, Streaking, and Spread Plates

Microbiologists rely on aseptic technique, dilution, colony streaking and spread plates for day-to-day experiments.

Microbiologists have many tools, but four relatively simple techniques are used by microbiologists daily, these are outlined here.

Aseptic technique or sterile technique is used to avoid contamination of sterile media and equipment during cell culture. Sterile technique should always be employed when working with live cell cultures and reagents/media that will be used for such cultures. This technique involves using flame to kill contaminating organisms, and a general mode of operation that minimizes exposure of sterile media and equipment to contaminants.



Serial Dilution: Example of Serial dilution of bacteria in five steps. The diluted bacteria were then spread plated. By Leberechtc (Own work) [GFDL (http://www.gnu.org/copyleft/fdl.html) and CC-BY-3.0 (http://creativecommons.org/licenses/by/3.0)], via Wikimedia Commons

When working with cultures of living organisms, it is extremely important to maintain the environments in which cells are cultured and manipulated as free of other organisms as possible. This requires that exposure of containers of sterilized culture media to outside air should be minimized, and that flame is used to "re-sterilize" container lids and rims. This means passing rims and lids through the flame produced by a Bunsen burner in order to kill microorganisms coming in contact with those surfaces. Sterile technique, in general, is a learned state-of-being, or mantra, where every utilization of any sterile material comes with the caveat of taking every precaution to ensure it remains as free of contaminants as possible for as long as possible. Heat is an excellent means of killing microorganisms, and the Bunsen burner is the sterile technician's best friend.

A serial dilution is the step-wise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A ten-fold serial dilution could be 1 M, 0.1 M, 0.01 M, 0.001 M… Serial dilutions are used to accurately create highly-diluted solutions as well. A culture of microbes can be diluted in the same fashion. For a ten-fold dilution on a 1 mL scale, vials are filled with 900 microliters of water or media, and 100 microliters of the stock microbial solution are serially transferred, with thorough mixing after every dilution step. The dilution of microbes is very important to get to microbes

In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.The streaking is done using a sterile tool, such as a cotton swab or commonly an inoculation loop. This is dipped in an inoculum such as a broth or patient specimen containing many species of bacteria.The sample is spread across one quadrant of a petri dish containing a growth medium, usually an agar plate which has been sterilized in an autoclave. Choice of which growth medium is used depends on which microorganism is being cultured, or selected for. Growth media are usually forms of agar, a gelatinous substance derived from seaweed.

Spread plates are simply microbes spread on a media plate. Microbes are in a solution, and can be diluted. They are then transferred to a petri dish with media specific for the growth of the microbe of interest. The solution is then spread uniformly through a number of possible means, the most popular is the use of sterile glass beads that are shook on top of the media, spreading the microbe-containing liquid evenly on the plate. Also common is the use of a bent-glass rod, often referred to as a hockey stick, due to its similar shape. The glass rod is sterilized and used to spread the microbe-containing liquid uniformly on the plate.

Special Culture Techniques

Many microbes have special growth conditions or require precautions to grow in a laboratory setting, leading to special culture techniques.

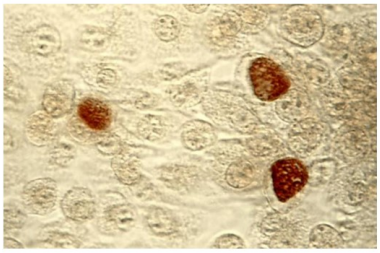
Microbiologists would prefer to use well-defined media to grow a microbe, making the microbe easier to control. However, microbes are incredibly varied in what they use as a food source, the environments they live in, and the danger levels they may have for humans and other organisms they may compete with. Therefore they need special nutrient and growth environments. To grow these difficult microbes, microbiologists often turn to undefined media which is chosen based on price and more-so in this case by necessity as some microorganisms have never been cultured on defined media. Some special culture conditions are relatively simple as demonstrated by microaerophile.

A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (~20% concentration). Many microphiles are also capnophiles, as they require an elevated concentration of carbon dioxide. In the laboratory they can be easily cultivated in a candle jar. A candle jar is a container into which a lit candle is introduced before sealing the container's airtight lid. The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar. Many labs also have access directly to carbon dioxide and can add the desired carbon dioxide levels directly toincubators where they want to grow microaerophiles.

Candle jar: A candle is lit in a jar with a culture plate. The lid is put on, as the burns it increases the carbon dioxide levels in the jar.

Animals can often be used to culture microbes. For example, armadillos are often used in the study of leprosy. They are particularly susceptible due to their unusually low body temperature, which is hospitable to the leprosy bacterium, Mycobacterium leprae. The leprosy bacterium is difficult to culture and armadillos have a body temperature of 34°C, similar to human skin. Likewise, humans can acquire a leprosy infection from armadillos by handling them or consuming armadillo meat. Additionally, Syphillis which is caused by the bacteria *Treponema pallidum* is difficult to grow with defined media, so rabbits are used to culture *Treponema pallidum*. *Treponema pallidum* belongs to the Spirochaetesphylum of bacteria.

To date Spirochaetes are very difficult if not impossible to rear in a controlled laboratory environment. This also includes other human pathogens like the bacterium that causes Lyme disease. Using animals to culture human-pathogens has problems. First, the use of animals is always difficult for technical and ethical reasons. Also, a microbe growing on animal other than a human may behave very differently from how that same microbe will behave on a human. Some human pathogens are grown directly on cells cultured from humans. Exemplified by the bacteria *Chlamydia trachomatis*, the bacteria responsible for the sexually transmitted infection (STI) in humans known as Chlamydia. As *Chlamydia trachomatis* only grows in humans. The human cell culture known as McCoy cell culture is used to culture this bacteria.



Chlamydias bacteria group: Light microscope view of cells infected with chlamydiae as shown by the brown inclusion bodies.

Biosafety Level 1: This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, with minimal potential hazard to laboratory personnel and the environment.

Biosafety Level 2: This level is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the

environment. It includes various bacteria and viruses that cause only mild disease to humans or are difficult to contract via aerosol in a lab setting such as chlamydia.

Biosafety Level 3: This level is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease after inhalation. It includes various bacteria, parasites, and viruses that can cause severe to fatal disease in humans, but for which treatments exist (eg. yellow fever).

Biosafety Level 4: This level is reserved for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents that cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as Bolivian and Argentine hemorrhagic fevers, Marburg virus, and the Ebola virus. Very few laboratories are biosafety level 4.

Enrichment and Isolation

The most common growth media for microorganisms are nutrient broths and agar plates; specialized media are required for some microorganisms. Some, termed *fastidious organisms*, require specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells.

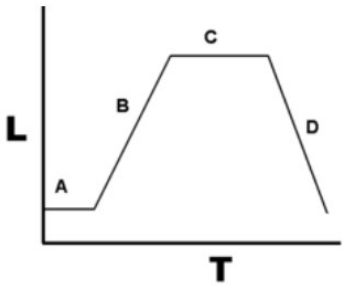
Growth media: defined vs. undefined

An important distinction between growth media types is that of defined versus undefined media.

A defined medium will have known quantities of all ingredients. For microorganisms, this consists of providing trace elements and vitamins required by the microbe, and especially, a defined source of both carbon and nitrogen. Glucose or glycerol is often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources.

An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.

Growth phases



During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

Exponential phase (sometimes called the log or logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population.Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

The stationary phase is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids.At death phase, bacteria run out of nutrients and die.

Culture

Batch culture is the most common laboratory-growth method in which bacterial growth is studied, but it is only one of many. The bacterial culture is incubated in a closed vessel with a single batch of medium.

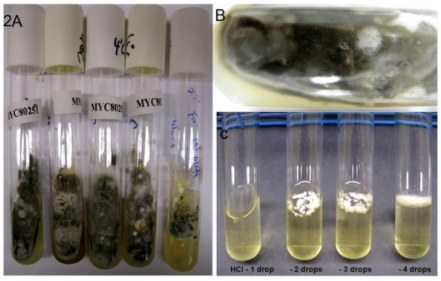
In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a *chemostat*, also known as an open or continuous culture: a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related

devices include *turbidostats* and *auxostats*. Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria.

In a synecological culture, a true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual.

Pure Culture

A pure culture is a population of cells or multicellular organisms growing in the absence of other speciesor types.



Selective Media: Geomyces destructans in culture from bat tissues. (A) Original culture tubes of Sabouraud agar supplemented with nine antibiotics and incubated at 4°C for six- or eight-weeks; notice the profuse growth of G. destructans strains. (B) Some fungal contamination on individual isolates was visible as depicted in the close-up of a culture tube. (C) Enrichment and recovery of pure fungal colonies by treating a culture contaminated with bacteria with hydrochloric acid.

Microbial cultures are foundational and basic diagnostic methods used extensively as a research tool in molecular biology. It is often essential to isolate a pure culture of microorganisms. A pure (or axenic) culture is a population of cells or multicellular organisms growing in the absence of other species or types. A pure culture may originate from a single cell or single organism, in which case the cells are genetic clones of one another. For the purpose of gelling the microbial culture, the medium of agarose gel (agar) is used. Agar is a gelatinous substance derived from seaweed. A cheap substitute for agar is guar gum, which can be used for the isolation and maintenance of thermophiles.

Microbiological cultures can be grown in petri dishes of differing sizes that have a thin layer of agar-based growth medium. Once the growth medium in the petri dish is inoculated with the desired bacteria, the plates are incubated at the best temperature for the growing of the selected bacteria (for example, usually at 37 degrees Celsius for cultures from humans or animals or lower for environmental cultures). Another method of bacterial culture is liquid culture, in which the desired bacteria are suspended in liquid broth, a nutrient medium. These are ideal for preparation of an antimicrobial assay. The experimenter would inoculate liquid broth with bacteria and let it grow overnight (they may use a shaker for uniform growth). Then they would take aliquots of the sample to test for the antimicrobial activity of a specific drug or protein (antimicrobial peptides). As an alternative, the microbiologist may decide to use static liquid cultures. These cultures are not shaken and they provide the microbes with an oxygen gradient.

Preserving Bacterial Cultures

Bacteria can be stored for months or years if they are stored at -80C and in a high percentage of glycerol.

Bacteria in liquid media: An erlenmeyer containing a bacterial culture. Bacteria that have been preserved in glycerol stocks can be grown overnight in liquid media to promote propagation.

Three species of bacteria, Carnobacterium pleistocenium, Chryseobacterium greenlandensis, and Herminiimonas glaciei, have reportedly been revived after surviving for thousands of years frozen in ice. As a practical matter, as a researcher, you will want to preserve your selected bacteria so you can go back to it if something goes wrong.

Whenever you successfully transform a bacterial culture with a plasmid or whenever you obtain a new bacterial strain, you will want to make a long-term stock of that bacteria. Bacteria can be stored for months and years if they are stored at -80C and in a high percentage of glycerol.

In order to ensure a pure culture is being preserved, pick a single colony of the bacteria off a plate, grow it overnight in the appropriate liquid media, and with shaking. Take the overnight culture and and mix an aliquot with 40% glycerol in sterile water and place in a cryogenic vial. It is important to label the vial with all the relevant information (e.g. strain, vector, date, researcher, etc.). Freeze the glycerol stock and store at -80C. At this point you should also record the strain information and record the location.

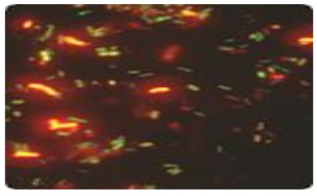
While it is possible to make a long term stock from cells in the stationary phase, ideally your culture should be in logarithmic growth phase. Certain antibiotics in the medium should be removed first as they are supposedly toxic over time, e.g. Tetracycline. To do this, spin the culture down and resuspend it in the same volume of straight LB medium.

The FISH Technique

FISH is a hybridization technology which allows the labeling of target RNAs with a fluorescent probe.

FISH (fluorescence in situ hybridization) is a cytogenetic technique developed by biomedical researchers in the early 1980s. It is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes bind to those targets that show a high degree of sequence complementarity. FISH can be used to detect RNA or DNA sequences of interest. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets, including mRNAs, in cells. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues.

Central to FISH are the use of probes. The probe must be large enough to hybridize specifically with its target but not so large as to impede the hybridization process. They are anti-sense to the target mRNA or DNA of interest, thus they hybridize to targets. The probe can be tagged directly with fluorophores, or with targets for flourescently labelled antibodies or other substrates. Different types of tags can be used, therefore different targets can be detected in the same sample simultaneously (multi-colour FISH). Tagging can be done in various ways, such as nick translation, or PCR using tagged nucleotides. Probes can vary in length from 20 to 30 nucleotides to much longer sequences.

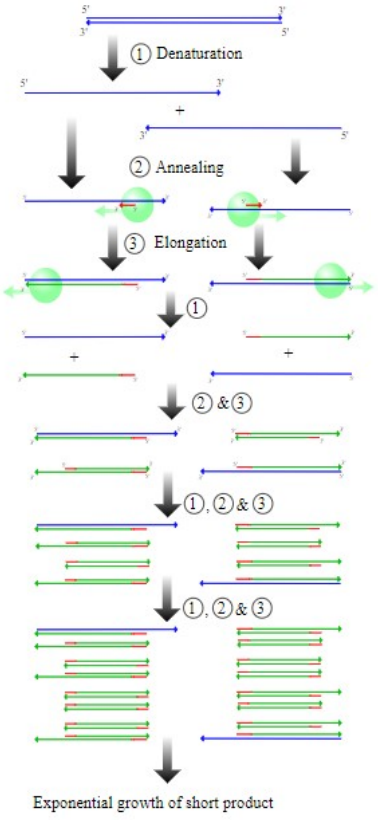


Dual label FISH image: Here is an example of FISH being used to differentiate Bifidobacteria (red) and other bacteria (green)

FISH is often used in clinical studies. If a patient is infected with a suspected pathogen, bacteria from the patient's tissues or fluids, are typically grown on agar to determine the identity of the pathogen. Many bacteria, however, even well-known species, do not grow well under laboratory conditions. FISH can be used to directly detect the presence of the suspect on small samples of the patient's tissue. FISH can also be used to compare the genomes of two biological species, to deduce evolutionary relationships. A similar hybridization technique is called a zoo blot. Bacterial FISH probes are often primers for the 16s rRNA region. FISH is widely used in the field of microbial ecology, to identify microorganisms. Biofilms, for example, are composed of complex (often) multi-species bacterial organizations. Preparing DNA probes for one species and performing FISH with this probe allows one to visualize the distribution of this specific species within the biofilm. Preparing probes (in two different colors) for two species allows to visualize/study co-localization of these two species in the biofilm, and can be useful in determining the fine architecture of the biofilm.

Coupling Specific Genes to Specific Organisms Using PCR

PCR allows for the amplification and mutation of DNA and allowing researchers to study very small samples.Polymerase chain reaction (PCR) is a useful technique for scientists, because it allows for the amplification and mutation of DNA. Through PCR, small quantities of DNA can be replicated by orders of magnitude, not only essentially preserving the sample if successful, but allowing for study on a much larger scale.. Without PCR, the studies we perform would be limited by the amount of DNA we were able to isolate from samples. Through PCR, the original DNA is essentially limitless, allowing scientists to induce various mutations in different genes for further study.



Polymerase chain reaction: a schematic of the polymerase chain reaction

Through site-directed mutagenesis or customized primers, individual mutations in DNA can be made. By changing the amino acids transcribed from DNA through individual mutations, the importance of those amino acids with respect to gene function can be analyzed. However, this process can be difficult, particularly when genes act in concert (with varying expression with respect to gene activity). The length of time it takes to run a successful PCR and perform other techniques before additional studies can be done (protein expression, isolation, and purification, for example), makes biochemical research time-consuming and difficult. However, PCR, coupled with other biochemical techniques, allows us to analyze the very core of organisms and the processes by which they function. Common PCR protocols in labs today include knockout genotyping, fluorescence genotyping and mutant genotyping. Researchers can use PCR as a method of searching for genes by using primers that flank the target sequence of the gene along with all other necessary components for PCR. If the gene is present, the primers will bind and amplify the DNA, giving a band of amplified DNA on the agarose gel that will be run. If the gene is not present, the primers will not anneal and no amplification will occur.

The ability to identify specific genes to specific organisms has increased the use of PCR and has allowed it to be more specific and eliminate the possibility of cross contaminants. The identification of specific genes to specific organisms has important medical diagnostic value.

PCR is a reliable method to detect the presence of unwanted genetic materials, such as infections and bacteria in the clinical setting. It can even allow identification of an infectious agent without culturing. For example, in diagnosis of diseases like AIDS, PCR can be used to detect the small percentage of cells that are infected with HIV by utilizing primers that are specific for genes specialized to the HIV virus. PCR can reveal the presence of HIV in people who have not mounted an immune response to this pathogen, which may otherwise be missed with an antibody assay). Additionally, PCR is used for identifying bacterial species, such as *Mycobacterium tuberculosis* in tissue specimens. With the use of PCR, as few as 10 bacilli per million human cells can be readily detected. The bacilli are identified by using *Mycobacterium tuberculosis* specific genes.

Chemical Assays, Radio isotopic Methods, and Microelectrodes

There are numerous tests and assays available that are utilized to aid in bacterial identification in a variety of settings.
Within the field of microbiology, there are specific tests or assays utilized to quantitatively and qualitatively measure microorganism components. These assays are often utilized to aid in bacterial identification. Three major types used for this purpose include chemical assays, radio isotopic methods and the use of micro electrodes. The following is an overview of these methodologies.

Chemical Assays

Chemical assays are utilized to identify and determine chemical components within a microorganism. Many of these assay's test for specific cellular components and may have overlap with chemical analysis, which focuses on exact chemical composition.

Disadvantages of culture media

1. Other Antimicrobial Drugs
2. Drug Resistance
3. Culturing Viruses

4. Genetic Engineering Products
5. Culturing Bacteria

1. Other Antimicrobial Drugs

Antifungal Drugs The development of antifungal drugs focuses on the classes of mycotic diseases for which fungi are responsible. Learning Objectives Compare and contrast the mechanisms of action for: polyene, azole,allylamine and echinocandin antifungals Key Takeaways Key Points The various classes of antifungal drugs exploit the unique fungal structure.

2. Drug Resistance

Mechanisms of Resistance Development of microbial resistance to antimicrobial agents requires alterations in the microbe's cell physiology and structure. Learning Objectives Describe the mechanisms bacteria use to develop antimicrobial resistance and the factors that can lead to it Key Takeaways Key Points Antimicrobial resistance can be mediated by the environment or the microorganism itself.

3. Culturing Viruses

Batch Culture of Bacteriophages Bacteriophage cultures require host cells in which the virus or phage multiply. Learning Objectives Define the reasons for, and ways to batch culture bacteriophages Key Takeaways Key Points A bacteriophage is a type of virus that infects bacteria. It does so by injecting genetic material

4. Genetic Engineering Products

Biotechnology is the use of biological techniques and engineered organisms to make products or plants and animals that have desired traits. Learning Objectives Describe the historical development of biotechnology Key Takeaways Key Points For thousands of years, humankind has used biotechnology in agriculture, food production, and medicine.

5. Culturing Bacteria

Culture media is the food used to grow and control microbes. Learning Objectives Classify culture media Key Takeaways Key Points Culture media contains the nutrients needed to sustain a microbe. Culture media can vary in many ingredients allowing the media to select for or against microbes.

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