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CULTURE MEDIA

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ABSTRACT

Culture media is a growth medium made to meet the nutritional needs of certain microbes for reproduction and growth processes. The requirements for a good culture media are that it can provide sufficient nutrients to the microbes, there must be space for oxygen or other gases according to the needs of the microbes, and has the appropriate humidity. The composition of the culture media is developed according to the ability of bacteria/ other microorganisms to use these elements. Several characteristics of culture media that are important for microbial growth include nutrients such as carbon, nitrogen, inorganic phosphate, sulfur, inorganic minerals, water, and vitamins. Culture media are available in various types and classifications. Selection of the right culture media is needed to provide maximum microbial culture results. The key steps in the manufacture of media are initial preparation, rehydration, sterilization, addition of supplements, filling, labeling, and secondary sterilization. The method commonly used for the isolation of microorganisms on culture media is the streak-plate method. Quality control is carried out to ensure quality and determine the minimum requirements for the preparation of culture media to be used for microbiological analysis, as well as determine the criteria and methods for testing the performance of culture media.

Keywords: Culture Media, Terms, Classification, Quality Control.

I. INTRODUCTION

Bacterial culture is an essential method for the study of virulence and susceptibility to antibiotics.[1] Culture is also the first method developed to study the human microbiota using artificial media that allows the growth and isolation of bacteria.[2] Culture media are growth media made to meet the nutritional needs of microbes specific for reproduction and growth. Media that are often used for bacterial culture include liquid media and solid media. In liquid media, microbial growth will make the media appear more cloudy. As for the culture process on solid media, the sample is inoculated on the culture medium, which will then grow as colonies (in bacteria and yeast) or as filaments (fungi). Colony characteristics, such as color, shape, and size, are very useful in determining the type of pathogen. The samples used can come from the environment such as soil, air, food, or from clinical specimens such as blood, saliva, feces, and cerebrospinal fluid.[3]

The discovery of culture media was the beginning of the rapid advances in microbiology in the nineteenth century. In 1881, Robert Koch demonstrated optimal bacterial growth when incubated in a broth consisting of fresh beef serum or meat extract. However, the use of liquid culture media did not produce pure bacterial cultures. Koch found a way to make solid media, by replacing gelatin with agar so that it could isolate bacteria. In 1887, Julius Richard Petri replaced the flat glass plate that is usually used for bacterial culture media with a circular container, and is still used today. This allows it to observe the colony and reduce contamination.[2]

II. CHARACTERISTICS AND CONDITIONS OF CULTURE MEDIA

It is necessary to pay attention to the elements that support the success of the culture media, namely the type of nutrient selected, atmospheric conditions, temperature, and incubation duration.[1] The requirements for a good culture medium are that it can provide sufficient nutrients to microbes, there must be room for oxygen or other gases according to the needs of microbes, and has the appropriate humidity. In addition, to support microbial growth, it is necessary to have an appropriate pH setting, have an appropriate temperature, be free from

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interference with bioburden (the initial population of living microorganisms in a product), and be free from contamination.[5]

Several characteristics of culture media that are important for microbial growth include nutrients such as carbon, nitrogen, inorganic phosphate, sulfur, inorganic minerals, water, and vitamins. These ingredients are naturally available in meat infusion. Beef extract or yeast can often replace meat infusion in culture media. The addition of peptone, a product of protein metabolism, can provide an additional supply of nitrogen and carbon. The temperature also needs to be adjusted to the microbes. For example, in mesophilic bacteria and fungi, optimal growth occurs at 25-40 ^{o}C; thermophilic organisms grow optimally at temperatures above 45^{o}C; and psychrophilic organisms require temperatures below 20^{o}C. Organic pathogens in the human body are generally mesophilic.[2,5]

III. INGREDIENTS AND COMPOSITION OF CULTURE MEDIA

The composition of the culture media is developed according to the ability of bacteria/other microorganisms to use these elements. Some of the required compositions and sources are shown in Table 1.[5]

Table 1. Composition of culture media and alternative sources.[5]

Composition	Sources
Amino-Nitrogen	Peptone, protein hydrolysate, extract and infusion
Growth factor	Blood, serum, yeast extract or vitamins, NAD (nicotinamide adenine dinucleotide)
Source of energy	Sugar, alcohol, and carbohydrates
Salt buffer	Phosphate, acetate, and citrate
Mineral salts and metal	Phosphate, sulphate, magnesium, calcium, iron
Selective agents	Chemical, antimicrobial, and staining agents
Indicator dye	Phenol red, neutral red
Gelling agent	Agar, gelatin, alginate, silica gel

There are many variations of materials for making culture media, some of the materials that are often used are[6–9]:

a. Agar

Agar is the solidifying agent most often used to make culture media. Agar is a polysaccharide made from marine algae extracts to make solid media and semi-solid media.

b. IsoVitaleX™

IsoVitaleX™ (BD) is a chemical additive used for the cultivation of fastidious bacteria. This reagent is used as a substitute for yeast concentrate which serves as a nutritional supplement for the growth of bacteria including gonococci and Haemophilus. IsoVitaleX™ consists of a blend of Vitamin B12, L-Glutamine, adenine, guanine hydrochloride p-Aminobenzoic acid, nicotinamide adenine dinucleotide, thiamine pyrophosphate, ferric nitrate, thiamine hydrochloride, L-cysteine hydrochloride, L-cysteine, and dextrose.

c. Nicotinamide Adenine Dinucleotide (NAD)

The addition of NAD to bacterial culture media provides an important role in metabolism as a coenzyme in redox reactions and can serve as a substrate for bacterial DNA ligase.

d. Peptone

Peptone is a water-soluble protein derivative and is widely used in bacterial culture media. Peptone is used in bacterial media as a source of organic nitrogen and is often used in serum-free media.

e. Yeast extract

Yeast extract was prepared from the autolyzed and water-soluble extraction of Saccharomyces cerevisiae yeast cells. During autolysis, yeast's endogenous digestive enzymes break down protein content into peptides and amino acids that can be used by bacteria as a nitrogen source. In addition, yeast extract provides an important source of water-soluble B-complex vitamins, carbohydrates, and free glutamic acid.

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f. Albumin-Dextrose-Catalase (ADC) and Oleic acid-Albumin-Dextrose-Catalase (OADC)

Enrichment ADC was used in Middlebrook 7H9 medium for selective growth of mycobacteria. This ingredient is formulated with sodium chloride, bovine albumin, dextrose, and catalase. The addition of dextrose provides an additional source of energy. Both albumin and catalase have a protective role by binding to free fatty acids that may be toxic to mycobacteria and cleaning the environment of toxic peroxides. Meanwhile, OADC is an enrichment supplement that is added to Middlebrook 7H10 agar which also plays a role in the culture of mycobacteria. This additive uses the same reagents as ADC but there is an addition of oleic acid. The addition of OADC makes the growth of Mycobacterium species faster and stronger.

g. Sodium pyruvate

The addition of sodium pyruvate in the bacterial culture medium provides a source of energy and a carbon skeleton for anabolic processes. This reagent has also been shown to provide protection against hydrogen peroxide.

h. Dextrose

Dextrose is a highly nutritional supplement used for the culture of fastidious organisms, older cultures, and cultures with small inoculums.

Some growth media used for certain bacterial species may require the addition of components that are not yet available in the base material. The following are some types of additional supplements used in the manufacture of culture media[6,10]:

a. Chemical supplements

- **Trace elements**

Trace elements are micronutrients required by microorganisms in small amounts. These nutrients consist of metal ions which are required by most microorganisms to survive because they generally act as cofactors for essential enzymatic reactions. Examples of trace elements needed for bacterial nutrition are zinc, copper, manganese, molybdenum, and cobalt.

- **Salt**

Various types of salts, such as magnesium, calcium, iron, and potassium salts, are needed for bacterial growth because they provide the main elements that can function as cofactors for certain enzymatic reactions. In addition to the functions mentioned above, calcium and iron are the building blocks for the main components of endospores and cytochromes. The presence of salt, particularly sodium chloride, in the medium also helps maintain the osmolality of the medium.

- **Glucose**

Most microbial culture media need to be supplemented with glucose as a carbohydrate source. In addition to its nutritional role, glucose can also be added as a means to suppress the performance of several enzyme sequences that can be induced in several different bacterial species through inhibition of cyclic AMP (cAMP) synthesis. The activity of adenylate cyclase, an enzyme required for cAMP synthesis, is blocked in the presence of glucose. In bacterial species such as Escherichia coli, this inhibition requires the use of glucose by the cells as the primary energy source rather than metabolizing other energy sources.

- **Blood**

Blood is often added to the growth medium to enhance the culture of very fastidious microbial species. Examples of such media are Tryptic Soy Agar and Dextrose Agar, both of which are often supplemented with 5% defibrillated sheep blood.

b. Antibiotic

Antibiotics can be added to the culture media, so that only certain bacterial variants can grow. This type of media is often used for the selection of strains that have antibiotic resistance properties, or those that are genetically engineered. Some are used for the elimination of Gram-positive bacteria, such as penicillin G, bacitracin or vancomycin, and some are used for the elimination of Gram-negative bacteria, such as colistin or polymyxin B, and some are used for the elimination of fungi and yeasts, such as Amphotericin B, cycloheximide, nystatin. The addition of antibiotics can reduce the shelf life of the media compared to the basic media. To prolong shelf life, media containing antibiotics should be stored at 2-8°C in a dark place.

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c. Serum

Serum can function as a source of growth factors, proteins, vitamins, hormones, carbohydrates, lipids, amino acids, minerals, and trace elements. In addition, serum can function as a pH buffer and can inactivate proteolytic enzymes. The exact composition of serum is unknown and varies from group to group. Serum from rabbits, horses, bovine fetuses, and calves is used to support the growth of several bacterial species in culture.

IV. CLASSIFICATION OF CULTURE MEDIA

1. Based on Media Form

a. Liquid media

The liquid or liquid media that is widely used is broth or broth. All components needed for growth media were dissolved in water, for example: peptone water, nutrient broth, Tarozzi, Tryptic soy broth, and phenol red carbohydrate broth. The process of bacterial culture with broth media is carried out in test tubes or flasks (Figure 1). After the sterilization and inoculation process, microbial growth will make the media cloudy or cloudy.[3] The weakness of this media is that it cannot be identified directly by its morphology, while its advantage is that it can provide access to better nutrients for bacteria..[2]

Figure 1. Use of liquid media for microbial culture.[3]

b. Solid media

Solid media are initially liquid media which are added with a solidifying agent such as agar. Agar is a cell wall polysaccharide derived from red algae. Like gelatin, agar melts when heated and hardens when cooled below 40°C. After sterilization of the nutrient agar medium, the solution will be poured onto a culture plate and allowed to cool and harden (Figure 2). Examples of solid media are carrot media, potato media, nutrient agar, blood agar, McConkey agar, and chocolate agar. After inoculation, specimens on agar media will grow as colonies (bacteria and yeast) or filaments (fungi). Solid media allows the user to see the differences in bacterial species through the resulting colonies. However, the weakness of solid media is that access to nutrients is more limited. Media with high gel content such as agar will form smaller colonies than media with lower gel content due to reduced nutrient flow and toxin removal.[2,11]

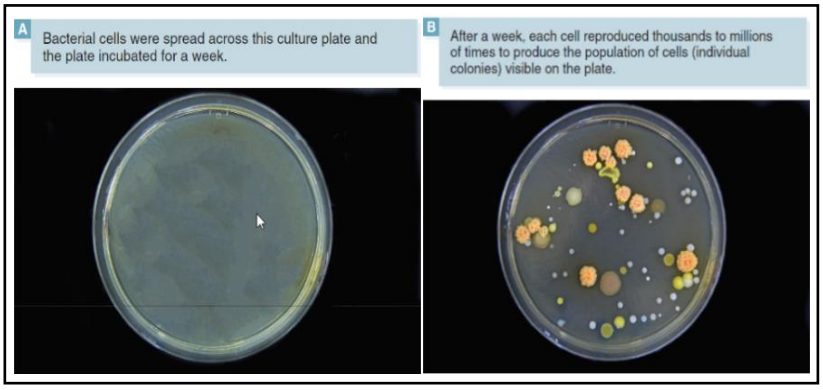

Figure 2. Use of solid media for microbial culture.[3]

c. Semi solid media

Semi-solid media, the same as solid media but the texture is semi-solid. The added compactor is less than half solid medium while the composition is the same as the others, for example Sulfide Indole Motility (SIM) agar,

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Carry & Blair, Stuart's-Amies media, mannitol motility media. Semi-solid media are used to study the motility of microorganisms, distinguish motile and non-motile bacterial strains, and grow microaerophilic bacteria.

2. Based on Media Composition

a. Simple Medium

It is a general purpose medium that supports the growth of non-fastidious microbes, and is mainly used for the isolation of microorganisms, for example nutrient broth, peptone water, and nutrient agar.

b. Complex Medium

In complex medium, the chemical composition of the medium is not known with certainty. This type of media often contains biological reagents such as yeast extract and peptone, Tryptic soy broth and blood agar. Complex mediums usually provide a wide variety of growth factors which aid in the culture of unknown bacterial species and fastidious bacteria.[3,12]

c. Defined Medium

In a defined medium, the chemical composition of the medium is known with certainty. This type of media usually consists of pure biochemical substances, and is often used to study the minimal nutritional requirements of a microorganism, for example Czapek Dox Medium.[3,12]

3. Based on Media Function

a. General Media

Used in general, this means that this media can be overgrown by various types of microorganisms, both bacteria and fungi, for example Nutrient Agar (NA).

b. Selective Medium

Selective medium is designed to inhibit the growth of certain bacterial species and promote the growth of other targeted bacterial species by the addition of substances such as antibiotics, bile salts, and pH regulation. For example, a complex medium containing salt (NaCl) to select the growth of Staphylococcus aureus. Another selective medium, for example, is MacConkey agar which selects the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria. In addition, there is also a selective medium for Xylose Lysine Deoxycholate (XLD) agar which functions for the isolation of Salmonella species from clinical samples and from food. Other examples are Blood agar, Brain Heart infusion agar, SS Agar for Salmonella shigella, Thayer-Martin Selective Agar for the isolation of Neisseria pathogens, and Serum Tellurite Agar for the isolation of members of the Corynebacterium genus, particularly in the laboratory diagnosis of diphtheria, and Lowenstein Jensen agar for Mycobacterium tuberculosis. Table 2 shows examples of selective medium with bacteria commonly used for culture.[3,5,7,10,11]

The typical colony morphology for Neisseria gonorrhoeae is small, grayish white to colorless, and slimy, while Neisseria meningitidis is medium to large, blue-gray in color, and mucoid. Corynebacterium resistant to tellurite produce gray to black colonies on tellurite-containing media.[3,5,7,10,11]

Table 2. Selective Medium.[10]

S. No	Culture media	Inhibiting substances	Bacteria
1	Thayer Martin Agar	Contains antibiotics; vancomycin, colistin, and nystatin	Used for Neisseria gonorrhoeae
2	MacConkey's Agar	Contains bile salts	Used for Enterobacteriaceae members
3	Lowenstein Jensen Medium	Addition of malachite green	Used for M.tuberculosis
4	Mannitol Salt Agar	Contains 10% NaCl	Used to recover S.aureus
5	Crystal Violet Blood Agar	Contains 0.0002% crystal violet	Used for Streptococcus pyogenes
6	Thiosulfate citrate bile salts sucrose (TCBS) agar	Have elevated pH of about 8.5-8.6	Used for isolating Vibrio cholerae
7	Wilson and Blair's Agar	Addition of dye brilliant green	Used for recovering S. typhi
8	Potassium tellurite medium	Contains 0.04% Potassium tellurite	Used to recover C.diphtheriae
9	Pseudosel Agar (cetrimide agar)	Contains cetrimide (antiseptic agent)	Used to recover Pseudomonas aeruginosa
10	Salmonella-Shigella Agar	Contains bile salts, brilliant green, and sodium citrate	Used for the isolation of Salmonella, which causes typhoid

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c. Differential Medium

The principle of differential medium is to modify the basic nutrient medium by the addition of one or more chemicals that allow observers to distinguish between similar species based on their colony appearance or visible growth changes. Differential medium contains certain chemicals to show physiological or biochemical differences between organisms, for example Eosin Methylene Blue (EMB) agar, blood agar, mannitol salts agar, MacConkey agar, and Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar. In Figure 3 on EMB agar media, we can distinguish E. coli bacteria that produce a greenish glow (number 1) and E. aerogens bacteria which are pink (number 3).[3,12]

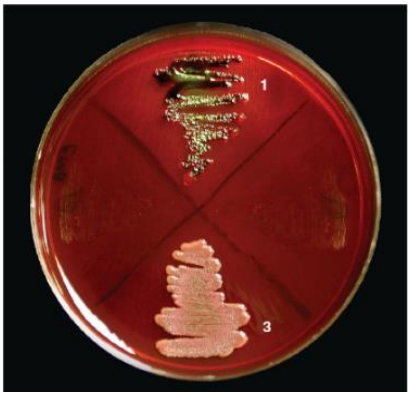

Figure 3. Example of differential medium EMB agar use.[3]

Figure 4 on blood agar media, we can distinguish the observed bacterial colonies from hemolysis of blood cells (alpha, beta, and gamma).

Figure 4. Example of differential medium blood agar use.[3]

Figure 5 on mannitol salts agar media, fermentation of mannitol by Staphylococcus aureus causes a yellow color change, in contrast to other staphylococcus species with negative coagulation, the color of the media does not change to yellow but pink.

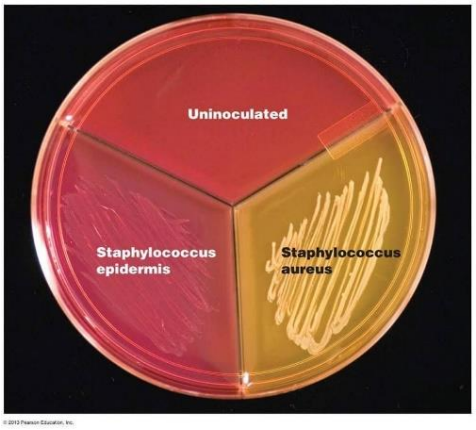

Figure 5. Example of differential medium mannitol salts agar use.[3]

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Figure 6 on MacConkey agar media, can distinguish gram-negative bacteria based on their lactose metabolism. Lactose fermenting bacteria, such as Eschericia coli, Klebsiella spp, Citrobacter, and Enterobacter form red-pink colonies, while non-lactose fermenting bacteria, such as Salmonella, Shigella, Proteus, Providencia, Pseudomonas, and Morganella form pale/colorless colonies.

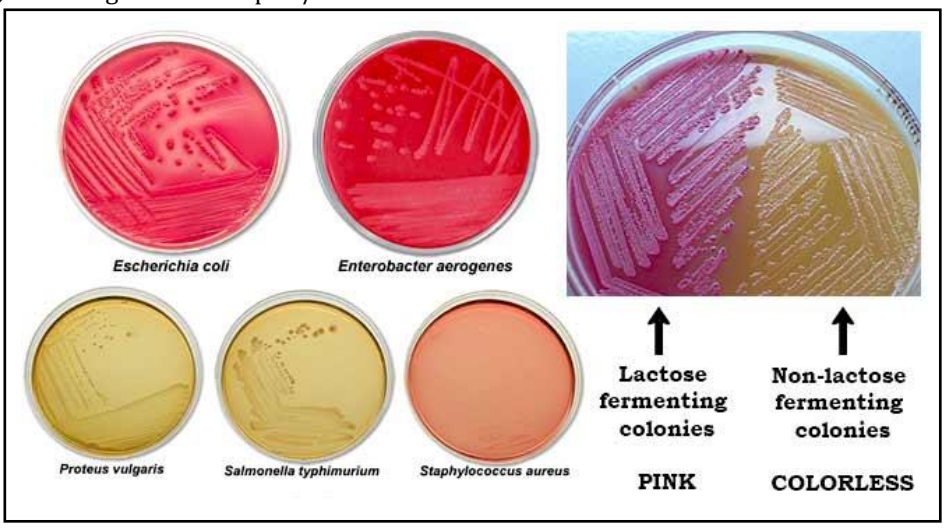

Figure 6. Example of differential medium MacConkey agar use.[3]

TCBS media containing sucrose can distinguish bacteria that ferment sucrose from those that do not ferment sucrose based on the color characteristics of the bacterial colonies, for example, V. cholerae which ferments sucrose with characteristic yellow colonies, and V. parahaemolyticus which does not ferment sucrose with characteristic green colonies (Figure 7).

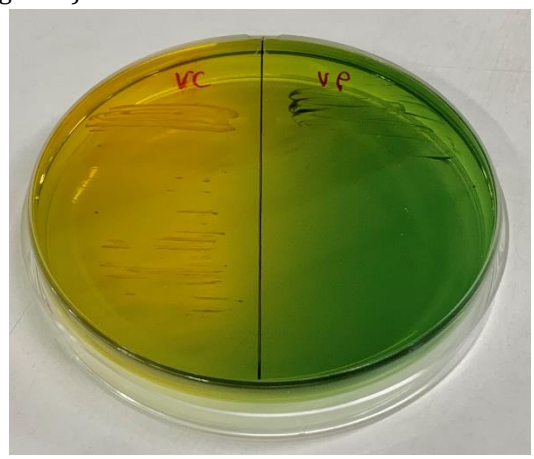

Figure 7. Example of differential medium TCBS agar use.[3]
Yellow color= *V. cholerae,* green color= *V. parahaemolyticus*

d. Enriched Nonselective Medium

Although many microorganisms grow well in standard complex medium, some organisms require enriched medium, which contains extra growth factors to promote cell growth and reproduction. Examples of enriched medium are blood agar containing standard nutrients plus 5% sheep red blood cells used for the growth of Salmonella enterica, chocolate agar for the growth of the bacteria that causes gonorrhea, Neisseria gonorrhoeae, requires hemoglobin powder as part of the growth medium (figure 8).[3,10]

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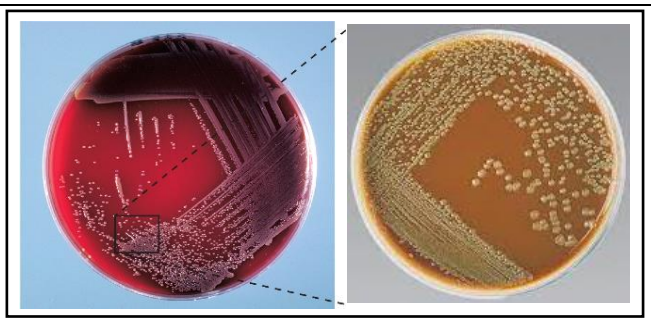

Figure 8. Example of enriched non selective medium use.[3]

A= blood agar, B=chocolate agar

e. Specialized Medium

Specialized medium or special media, made for the detection of certain organisms that are fastidious or that often appear in a mixture of many organisms. These bacteria have special complex nutritional requirements so they cannot grow on ordinary laboratory culture media. In addition, the growth tends to be slower.[7,10]

V. MAKING OF CULTURE MEDIA

The key steps in the manufacture of media are: initial preparation, rehydration, sterilization, addition of supplements, filling, labeling, and secondary sterilization.[11,13]

1. Initial preparation

For the preparation of broth/broth media, the broth can be poured into the required container (usually a glass bottle) prior to sterilization. The use of an accurate casting tool is necessary in this process. Quality control needs to be carried out by regularly checking the volume ejected, by measuring the volume or by verifying the weight. For the preparation of media plates, generally need to be sterilized, cooled, then poured into a petri dish. The contents of the plate volume must be considered during pouring.[13,14]

2. Rehydration

Preparation of culture media requires components that need to be dissolved. The powder medium is rehydrated by mixing a number of media into the required volume of water. The water used must be fresh, and some media require warm water. It is important to maintain the homogeneity of the solution by mixing it. For media containing agar as a solidifying agent, rehydration is carried out by mild heating and stirring the water mixture with the media to dissolve it. In this process, the media must be kept at a temperature (95-100°C), and should only be allowed to boil for a short time (less than 1 minute) so that the media does not burn.[8,13]

3. Sterilization

Most media are sterilized by autoclaving or in an agar preparator for multiple media plates. For some special cultures, media sterilization is done by filtration.[13]

4. Addition of supplements

The addition of supplements to the media needs to be done after the sterilization process, when the media is placed in a water bath with a lower temperature.[2,13]

5. Filling

On agar media, the material that has been sterilized needs to be poured into a petri dish. The transfer process needs to be done very carefully to avoid contamination. To prepare the filling of the petri dish, the sterilized medium must be carefully warmed while in the liquid state (until the temperature reaches about 45-50°C, gel formation usually occurs between 32 and 40 °C) and then poured into the heated petri dish. Sterile petri dishes are usually 95-100 mm or 50-55 mm in diameter. Agar media is often not evenly distributed as it melts and requires mixing to ensure a uniform distribution. Filling activities can be done semi-automatically using dish filler. It is necessary to periodically monitor the filling volume for quality control. Monitoring is usually done by weighing the plate that has been filled. Plate filling should be carried out under unidirectional airflow to minimize contamination. It is still necessary to avoid contamination even though secondary sterilization is planned, such as after irradiation.[13,14]

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6. Labelling

After the sterilization and filling steps have been carried out, all sterile broth bottles and plates filled must be labeled, and the details recorded in a batch record. The label must contain the media type, batch number, expiration time, and storage conditions.[13]

7. Secondary sterilization

For certain media plates, additional sterilization steps can be performed. The most common method is irradiation by ionization using gamma rays.[13]

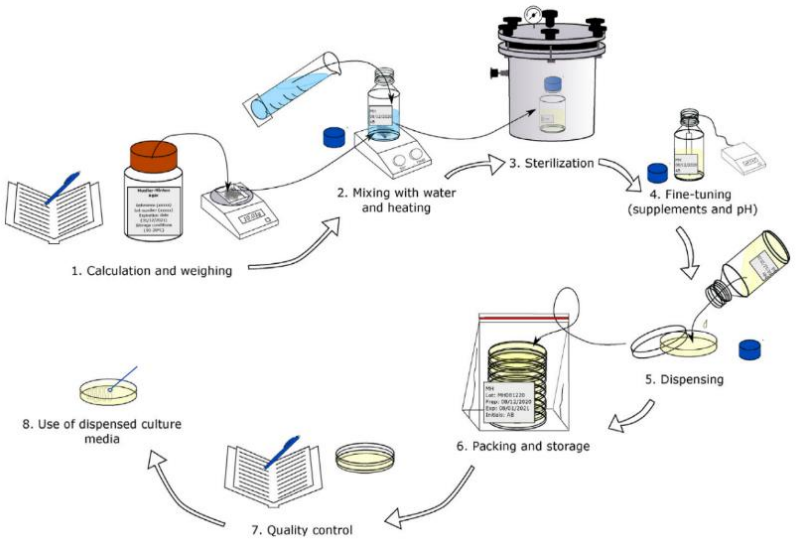

Figure 9. Illustration of the steps for making culture media.[15]

VI. ISOLATION OF MICROORGANISM IN CULTURE MEDIA

Microorganism isolation is an attempt to grow microorganisms outside their natural environment. The method commonly used for the isolation of microorganisms on culture media is the streak-plate method.[3] This method uses a single plate of sterile nutrient agar. A diluted sample of the mixed culture is taken with a sterile loop, and a series of streaks is made in one area of the plate. Next, the loop is heated and then touched to the first area, and a streak is made in the second area that brings some cells from the first area. Loops should always be sterilized/heated prior to contact with the inoculum. Similarly, streaks are created in the third and fourth regions, thereby separating the cells and they can grow into individual colonies. After 24-48 hours of incubation, discrete colonies will appear on the plate.[3,20]

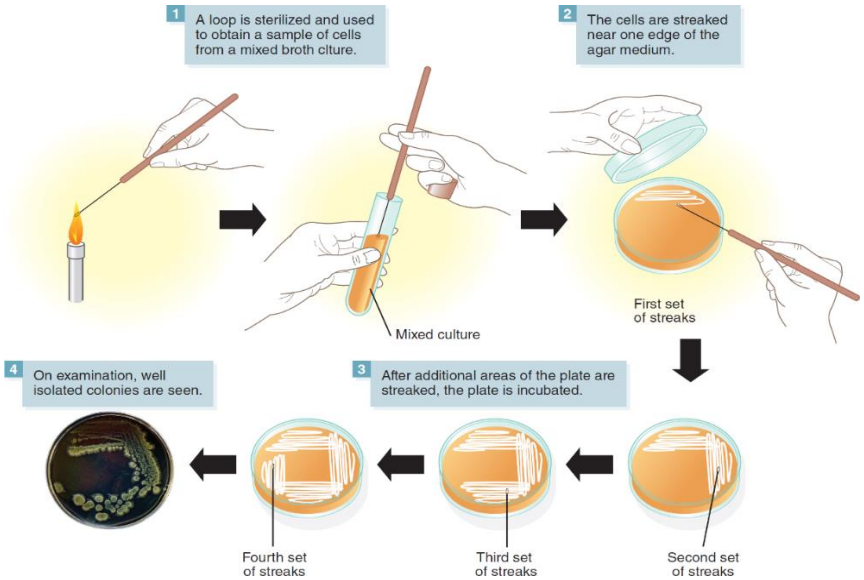

Figure 10. Streak-plate method steps.[3]

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VII. QUALITY CONTROL OF CULTURE MEDIA

Quality control is carried out to ensure quality and determine the minimum requirements for the preparation of culture media to be used for microbiological analysis, as well as determine criteria and methods for testing the performance of culture media. It is important to note that each batch of media undergoes some form of quality control before being released for general use to ensure that the results released by the microbiology laboratory are accurate. The test is usually carried out after all the preparation steps have been completed. Culture media quality control can be divided into two parts: physical characteristics and microbiological characteristics. Some of the errors that are often found in the quality control process and their possible causes can be seen in the table 3.[13,15]

1. Physical Characteristics

The tests performed to assess the physical characteristics of culture media vary depending on the type of culture medium. Some examples of tests carried out are[13,15]:

a) Color visual test

The color of the sterilized media should be compared with that of the unsterilized media and any color differences noted.

b) Clarity visual test

Media clarity should be checked for the presence of optical artifacts, such as crystallization.

c) Gel strength

The gel strength should not be too hard or too soft, but strong and usable.

d) pH of the media

This indicator is one of the most important chemical aspects because if the pH is outside the recommended range, it can cause growth inhibition for some microorganisms that want to be developed in the media.

e) Damage check

Plates and bottles must be checked for damage such as cracks and other defects.

2. Microbiologic Characteristics

The media sterility test is designed to detect microbial contamination during the media manufacturing process. Generally, 2% of the uninoculated media batch will be incubated. The temperature and time chosen for incubation of the sterility test will depend on the type of medium. For general media, a temperature of 30–35°C is used for 3 days. To pass the sterility test, the media must not show any microbial growth.[13,16]

To support growth, a panel of microorganisms is required to demonstrate the suitability of the medium for its intended use. The pharmacopoeia recommends that the desired microorganism should be traceable to a reputable pure culture collection, such as the American Type Culture Collection (ATCC). Pure cultures should be carefully stored in culture collections in the laboratory by ensuring that the storage temperature is low enough to avoid phenotypic variation and limit the number of subculture developments to less than five times. These pure microorganisms have generally been serially sub-cultured in national culture collection centers for decades and have been conditioned to grow on enriched media in the laboratory. In addition to pure culture, environmental isolates are generally also used in media testing. The organisms are designed to demonstrate that certain culture media will grow the types of microorganisms found in their manufacturing environment. For example, media used to test water will have a test panel that includes microbial isolates from water (such as Pseudomonas-associated bacteria), and media used for environmental monitoring will use bacteria that are temporary on human skin (such as Staphylococci).[13]

Table 3. Some errors during quality control and their possible causes.15.[15]

Errors	Possible causes
Clumps on dehydrated culture media	• Humidity is too high during storage • Containers open too long or not tightly closed • Culture media past storage age
Unsuitable pH	• pH meter is not calibrated • pH verification is carried out in an overheated medium (generally at

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	25°C) • Overheating: over-sterilization, heterogeneous mixtures, media stored at 50°C too long, repeated thawing or at too high a temperature • Poor water or container quality • Container contaminated with chemicals • Incomplete dissolving/mixing of media • Dehydrated media with improper storage or over shelf life
Incomplete solubility	• Inadequate use of water • Lack of heating/time to dissolve • Lack of soaking/mixing • The bottle is too small so it doesn't mix well
Darkening color, caramelization	• Overheating: over-sterilization, heterogeneous mixtures, media stored at 50°C too long, repeated thawing or at too high temperature • Incomplete dissolution of media
Gel shape is not perfect or to be too soft	• Incorrect proportion of product with water: weighing error or over dilution • To not dissolve well: poor mixing, too long storage at 50°C • Overheating of culture media, possibly at low pH or due to repeated thawing
Turbidity, precipitation	• Poor quality in dehydrated media, water or containers • Overheating: over-sterilization, heterogeneous mixtures, media stored at 50°C too long, repeated thawing or at too high a temperature • Inappropriate pH • Incomplete dissolving/mixing of media • Loss of water from the prepared culture medium due to evaporation
Poor microbial growth or loss of differential properties	• Improper quality control of organisms used • Overheating: over-sterilization, heterogeneous mixtures, media stored at 50°C too long, repeated thawing or at too high a temperature • Incomplete dissolving/mixing of media • There is an inhibitory substance in the water, container or inoculum • Inappropriate pH

3. Test Methods and Acceptance Criteria

a. Solid Media Test

For agar testing, a qualitative approach includes a simple streak subculture (spread plate). In this approach, the liquid culture is streaked with an inoculation loop to produce a single colony. Each segment of the agar plate can then be compared with the growth characteristics of the appropriate control plate (control media derived from a batch of media that was successfully released, having previously been assessed as having good growth promoting properties).[13,17]

b. Broth Media Test

Several laboratories tested the broth medium with a number of microorganisms and compared their growth over time with the control group. Some laboratories try a semiquantitative approach by creating a growth index ranging from little to abundant growth (usually using a +, ++, or +++ scale).[13]

c. Selective Media Test

A strain is required as a positive and negative control. Several microorganisms were used as positive or negative growth indicators to ascertain the growth characteristics for the selective medium being assessed. If a positive reaction is found, certain colony morphology, pigmentation, or diffusion of activity can be part of the criteria for acceptance of the culture media.[13,17]

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d. Assessment of Expiration Date

Each culture medium has certain storage conditions and expiration times, so the shelf life needs to be validated.[13,18]

VIII. CONCLUSION

Culture media is a growth medium that is made to meet the nutritional needs of certain microorganisms in the process of reproduction and growth. Since the 19th century, the discovery of culture media has made the development of the world of microbiology more advanced. A good culture medium must be able to meet all the basic needs for microbial growth. Some media materials have nutritional limitations, so that additional substances are supplemented that can support the function of the media.

Culture media are available in various types and classifications. There are several microorganisms that require selective media types, or are specific for certain microorganisms. Selection of the right culture media is needed to provide maximum microbial culture results. The process of making culture media is quite long and requires high accuracy so that it must be monitored with close supervision from microbiologists. In addition, before the culture media is circulated for public use, it is necessary to carry out a series of quality control processes or inspections by experts so that no failed products are widely circulated. Cultures need to be allowed to stand for several days to weeks for colony growth to be seen.

The process after the manufacture of culture media is the isolation and identification of microorganisms. Isolation was carried out to obtain pure cultures, while identification was carried out to determine the type of bacteria being cultured. Until now, the world of microbiology continues to grow, as well as culture technology. It is hoped that practitioners in the fields of microbiology and culture will continue to update their knowledge so that it can be adapted to daily practice.

IX. REFERENCES

[1] Lagier J, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev. 2015;28(1):208–36.

[2] Bonnet M, Lagier JC, Raoult D, Khelai S. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microbes New Infect. 2020;34(C).

[3] Pommerville JC. Fundamentals of Microbiology. 12th ed. Burlington, MA: Jones & Bartlett Learning; 2022.

[4] Austin B. The value of cultures to modern microbiology. Antonie Van Leeuwenhoek. 2017;

[5] Difco & BBL. Difco & BBL Manual: Manual of Microbiological Culture Media. 2nd editio. Zimbro M, Power D, Miller S, Wilson G, Johnson J, editors. Sparks, MD: Becton, Dickinson and Company;

[6] American Type Culture Collection. Bacteriology Culture Guide [Internet]. American Type Culture Collection. 2021 [cited 2022 Mar 5]. p. 28. Available from: https://www.atcc.org/resources/culture-guides/bacteriology-culture-guide

[7] Mobed A, Baradaran B, Guardia M de la, Agazadeh M, Hasanzadeh M, Rezaee MA, et al. Advances in detection of fastidious bacteria: From microscopic observation to molecular biosensors. TrAC - Trends Anal Chem [Internet]. 2019;113:157–71. Available from: https://doi.org/10.1016/j.trac.2019.02.012

[8] Atlas R, Snyder J. Reagents, Stains, and Media: Bacteriology. In: Jorgensen JH, Pfaller MA, editors. Manual of Clinical Microbiology. 11th editi. Washington, DC: ASM Press; 2015. p. 316–49.

[9] Das N, Triparthi N, Basu S, Bose C, Maitra S, Khurana S. Progress in the development of gelling agents for improved culturability of microorganisms. Front Microbiol. 2015;6(JUN):1–7.

[10] Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology. 9th ed. Philadelpia: Elsevier Inc.; 2021.

[11] Brown A, Smith H. Benson's Microbiological Applications: Laboratory Manual in General Microbiology. 13th ed. New York: McGraw-Hill Education; 2015.

[12] American Type Culture Collection. Introduction To Microbiology [Internet]. American Type Culture Collection. 2021 [cited 2022 Mar 5]. p. 40. Available from: https://www.atcc.org/resources/culture-guides/introduction-to-microbiology

[13] Sandle T. Pharmaceutical Microbiology: Essentials for Quality Assurance and Quality Control.

Woodhead P. Cambridge, UK: Woodhead Publishing Limited; 2016.

[14] Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev. 2015;28(1):237–64.

[15] Orekan J, Barbé B, Oeng S, Ronat JB, Letchford J, Jacobs J, et al. Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access. Clin Microbiol Infect. 2021;27(10):1400–8.

[16] Louis M, Leblanc L, Julian FAM. Introduction to Culture Media in Pharmaceutical Microbiology for Non-sterile Products. Pharm Microbiol Qual Assur Control. 2019;79–104.

[17] Hashim I. Microbiological Culture Media in Pharmaceutical Industry. Foster City, CA: OMICS Group eBooks; 2013. 1–15 p.

[18] Australian Society for Microbiology. Guidelines for Assuring Quality of Medical Microbiological Culture Media. 2012;(July):1–32.

[19] Jufri RF. Microbial Isolation. J La Lifesci. 2020;01(01):18–23.

[20] Sanders ER. Aseptic laboratory techniques: Plating methods. J Vis Exp. 2012;(63):1–18.

[21] Franco-Duarte R, Černáková L, Kadam S, Kaushik KS, Salehi B, Bevilacqua A, et al. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. 2019;7(5).