







ORIGINAL

Bacterial Culture Media in Clinical Microbiology: Advancing Beyond Selective Boundaries with Potential Implications for Cancer Prevention

Medios de cultivo bacteriano en microbiología clínica: superando los límites selectivos con posibles implicaciones para la prevención del cancer

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ABSTRACT

Clinical microbiology is crucial for the diagnosis of infectious disorders and it requires constant improvements in bacterial culture medium. It is done to attain changing diagnostic requirements and treatments. Cultural media were categorized into selective and non-selective, which is considered to be customized and more complex. The advancements of cultural media design is examined in this analysis with the objective of subtle formulations that enhance the diagnostic power. Progress with regards to microbial physiology has enabled the creation of specialized media that emulate host ecological systems and assisted the establishment of selective organisms. The review provides insights on the critical role that culture media plays in clarifying intricate microbial interactions and enhancing diagnostic chemical reactions in clinical microbiology as traverse of ever changing landscape is portrayed. The constant development assures the isolation of complex bacterial strains, delivering the dynamic character of microbiological research.

Keywords: Clinical Microbiology; Microbial Diagnostics; Nutrient; Culture-Based Diagnostics.

RESUMEN

La microbiología clínica es crucial para el diagnóstico de enfermedades infecciosas y requiere mejoras constantes en los medios de cultivo bacteriano. Esto se realiza para satisfacer las cambiantes necesidades diagnósticas y terapéuticas. Los medios de cultivo se clasificaron en selectivos y no selectivos, considerados personalizados y más complejos. En este análisis se examinan los avances en el diseño de medios de cultivo con el objetivo de formulaciones sutiles que mejoren la capacidad diagnóstica. Los avances en la fisiología microbiana han permitido la creación de medios especializados que emulan los sistemas ecológicos del huésped y han facilitado el establecimiento de organismos selectivos. Esta revisión proporciona información sobre el papel fundamental que desempeñan los medios de cultivo para esclarecer las complejas interacciones microbianas y mejorar las reacciones químicas diagnósticas en microbiología clínica, a través de un panorama en constante evolución. El desarrollo constante garantiza el aislamiento de cepas bacterianas complejas, lo que contribuye al dinamismo de la investigación microbiológica.

Palabras clave: Microbiología Clínica; Diagnóstico Microbiano; Nutrientes; Diagnóstico Basado en Cultivos.

INTRODUCTION

The environment that bacterial culture media provides for the development, isolation, and identification of bacteria renders them essential to clinical microbiology. The two primary classifications for cultural media are non-selective and selective. A wide variety of microorganisms may thrive on non-selective medium, although some kinds of bacteria are supported while others are inhibited in growing.⁽¹⁾ Clinical microbiology addresses the changing requirements of clinical diagnostics and developments in microbiology have prompted the creation of more complex and specialized culture media. The nineteenth-century evolution of microbiological was made feasible by the discovery of culture medium. The first technique developed to investigate the human microbiota was bacterial culture using a synthetic medium that permits bacterial growth and isolation.⁽²⁾ Differential media allows easy differentiation of similar bacteria according to certain physiological or biochemical traits. To assist microbiologists in identifying several species or strains, these media include substrates or markers producing significant variations in the appearance of colonies.

Supplementary nutrients are added to enriched medium to facilitate the development of selective microorganisms that might have intricate dietary requirements. For example, blood agar is a frequently used enriched medium that might be used to produce a variety of bacteria. It could be a challenging concern for developing over traditional substrates.⁽³⁾ The improvement of bacterial culture medium in clinical microbiology has expanded beyond conventional limits and established a dichotomy between selective and non-selective categories. Thus, the paradigm evolution is a result of the dynamic nature of microbiological research and the need for sophisticated instruments to handle challenging clinical situations. Culture media were traditionally classified as selective or non-selective, according to the capacity that promote the development of a certain range of microorganisms or facilitate the growth of a wide variety of bacteria.⁽⁴⁾

Although this categorization system has served as a basis, more developments have accelerated the creation of cultural media with a variety of functions. The advancement of special media offers a platform for discriminating between various microbe types based on their distinct biochemical properties. It is considered as one of the significant innovations that extend below simple selection. Bacteria with different metabolic profiles might be identified using these medium as they include indicators like pH-sensitive dyes or color changing chemicals.⁽⁵⁾ Another innovation is enriched media, which is designed to satisfy the nutritional demands of selective microbes. Microorganisms with particular nutritional needs may grow more effortlessly in such medium, because they include extra assets of blood or serum.⁽⁶⁾ The invention has rendered feasible to cultivate pathogens, which has improved the capacity to separate and identify germs that are therapeutically important.

Lab exploration has led to the establishment of culturomics, an innovative culture approach that expands the repertory of bacteria by using an extensive selection of growth conditions and medium.⁽⁷⁾ The approach illustrates the way of metagenomics and culturomics complement each other. The major goal is to improve diagnostic capabilities and enable better recognition of microbial pathogens by testing the limitations of existing selective and non-selected bacterial culture medium in clinical microbiology.

EVOLUTION OF CULTURE MEDIA: FROM THE FIRST BACTERIAL CULTURE

Microbiologists of the twenty-first century are aware with the culture media that emerged in the 1890s: peptones and agar were widely used and the suspensions were growing rapidly. Solid media was utilized in Petri plates.⁽⁸⁾ The creation of solid culture medium, such as agar plates, transformed the process of isolating and identifying microorganisms. Determining microbial variety and function required the isolation of individual bacterial colonies, which was made possible by the use of solid medium. Evolution of Culture Media was provided in table 1.

Table 1. Culture Media evolution in Clinical Microbiology

Era	Key Developments	Description
First Bacterial Culture (1890s)	Introduction of Peptones & Agar	Peptones and agar were widely used to grow bacterial cultures. Solid media, such as Petri plates, allowed for the isolation of individual colonies.
Early 20th Century	Selective & Differential Media	Development of selective media to promote or inhibit bacterial growth and differential media to distinguish bacteria based on biochemical properties.
Mid-Late 20th Century	Automation & High-Throughput Culture	Introduction of robotic systems and automated inoculation for handling large microbial libraries efficiently.

21st Century	Advanced Microbiology & Molecular Techniques	Clinical Use of automated systems, molecular diagnostics (PCR, DNA sequencing), and biochemical assays (ELISA, API systems) to improve bacterial identification and classification.
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SELECTIVE AND DIFFERENTIAL MEDIA: INNOVATIONS IN THE 20TH CENTURY

Scientists recognized microbiology, which is evolved to isolate specific types of bacteria and discern between closely related organisms, specialized culture medium were required. The notion of selective media is a mixture of substances that prevent certain germs from growing while encouraging the development of others appears. The ability to target and analyze certain microbial populations, this signaled the start of a new age in culture media. Differential media were developed in the 1940s and included chemicals or markers that made possible to visually distinguish between bacterial colonies depending on their biochemical properties.⁽⁹⁾ Based on colony color it made possible to distinguish between lactose-fermenting and non-fermenting bacteria.

HIGH THROUGHPUT OF CULTURE MEDIA AND AUTOMATION

Automating microbiology and developing high-throughput technologies have become more common in the twenty-first century. To monitor and inoculate hundreds of cultures, researchers have devised robotic devices.⁽¹⁰⁾ The microbial research field has advanced by making possible ways to identify new species or strains and screen enormous microbial libraries.

CLINICAL MICROBIOLOGY

The field of clinical microbiology is varied in terms of bacteria as it analyzes the techniques utilized to identify and describe species. It also includes a wide range of testing processes. In clinical microbiology, phenotypic and culture-based approaches remained to act as the main ways of identifying culture organisms, despite significant developments in testing techniques. To provide high-quality microbiology laboratory testing, error detection and correction are essential components. Microbiological testing is difficult because of the vast range of infections and testing methods.⁽¹¹⁾ All phases of testing (preceding, scientific, and post-analytical) are vulnerable to errors and faults in a single stage and are probable to coincide or produce errors in various steps (incorrect specimen collection, for example, can result in culture and identification). Quality control and quality assurance processes have decreased the occurrence of testing error in the clinical microbiology laboratory. Microorganisms persist to constitute obstacles in microbiological testing, despite developments in this field.

MICROBIOLOGY'S EMPIRICAL TECHNIQUE

Microscopic organism such as viruses, bacteria, fungi, and protozoa relied heavily on empirical techniques to expose the mysteries behind microbial world. Microscopy is a basic method used in microbiology. By using visible light to enlarge specimens, light microscopy enables scientists to examine the shape and organization of microorganisms. According to the properties of its cell walls, the approach is utilized alongside with staining techniques including Gram staining, a differential staining method that divides bacteria into Gram-positive and Gram-negative categories. Cultures are kept untainted using aseptic technique, a series of hygienic procedures. Microbiology has been transformed by molecular biology methods, which offers instruments for analyzing genetic material.⁽¹²⁾ One essential method is Polymerase Chain Reaction (PCR), which amplifies certain deoxyribonucleic acid (DNA) sequences and provides highly sensitive gene or microbe identification. Another effective technique is DNA sequencing, which offers information about the genetic code of microbes and helps with identification and categorization.

In microbiology, serological methods are crucial particularly for identifying and characterizing pathogens. An extensively used technique for determining, if antibodies or antigens are present in a sample is the Enzyme-Linked Immunosorbent Assay (ELISA). Tests using biochemistry can provide details about an organism's capacity for metabolism. These tests assist in differentiating between distinct microbial species by evaluating an organism's capacity to use particular substrates or generate particular products. By establishing a profile of a microorganism's metabolic characteristics, analytical profile index (API) systems standardized collection of biochemical tests, simplify the identification procedure.⁽¹³⁾ Microbial DNA is manipulated by using genetic procedures including gene cloning and recombinant DNA technologies. With the use of recombinant DNA technology, microorganisms are inserted to create engineered creatures with desired features. Gene cloning creates several copies of a gene for additional research. Table 2 provides a summary of key technological advancements in microbiology, highlighting their applications and examples

Table 2. Key Technological Advancements in Microbiology

Technique	Application	Examples
Microscopy	Visualization of microbial structures	Light Microscopy, Gram Staining

Molecular Methods	Genetic identification of microbes	PCR, DNA Sequencing
Serological Techniques	Detection of antigens/antibodies	ELISA
Biochemical Testing	Identification based on metabolism	API Systems
Genetic Engineering	Manipulating microbial DNA	Gene Cloning, Recombinant DNA Technology

MICROBIAL ANTHROPOLOGY

Numerous scientists examining that the bacteria have attempted to cultivate the bacterium on a meal or substance that it originated with varying amounts of efficiency. By disproving the idea of spontaneous generation, researches postulated that live organisms may originate from dead or decaying material.⁽¹⁴⁾ Industrial enzymes, medicines, and biofuels are produced in biotechnology with the help of culture microbiology. To increase the production of significant chemicals, large-scale cultures are developed and microorganisms are frequently selected or transformed on their capacity to manufacture certain compounds. To diagnose infectious disorders, harmful microorganisms must be isolated from clinical samples and identified. Figure 1 shows that microbial anthropology.

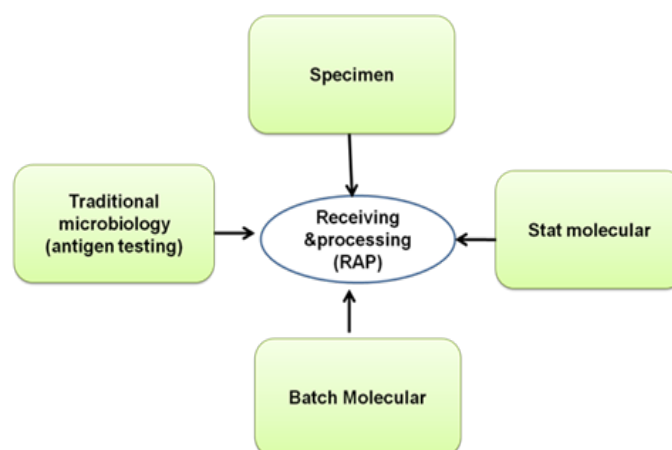


Figure 1. Microbial Anthropology

Enriched cultural medium

A nutrient-rich substrate known as an enriched culture media is made to promote the development of a variety of microorganisms by adding extra nutrients like blood, serum, or certain growth factors. The media makes easier to cultivate picky or nutrient-demanding microorganisms, facilitating their isolation and investigation in scientific settings.⁽¹⁵⁾ A media that has additional components that are necessary for bacterial growth is known as an enhanced culture medium. Microbiology utilizes water for hydrolysis, actions, and nutrient dissolution. Essential water conditions are required for specific microorganisms. Evaporation during agar incubation can lead to:

1. Water loss
2. colony shrinkage
3. inhibition of bacterial growth

Nitrogen sources in culture media

Culture media contain a variety of nitrogen-rich substances, essential for bacterial protein production. Nitrogen is supplied in utilizable forms.⁽¹⁶⁾ Table 3 presents the various organic and inorganic nitrogen sources utilized in bacterial culture media to support growth and metabolism.

Table 3. Organic and Inorganic Forms of Nitrogen Sources in Bacterial Culture Media	
Organic Forms	Inorganic Forms
Yeast extract	Nitrates
Casein hydrolysate	Ammonium salts
Soy peptone	Nitrites
Meat extract	Urea
Gelatin hydrolysate	Ammonia

The adaptability of nitrogen sources allows bacteria to maximize protein synthesis, crucial for their growth and abundance in lab cultures.

Listeria monocytogenes:

1. A Gram-positive, chemotrophic bacterium
2. A facultative anaerobe, capable of surviving with or without oxygen
3. Uses oxidative metabolism for energy production from organic substances (e.g., sugars)
4. Its adaptability enhances pathogenicity, leading to foodborne listeriosis

Biosynthetic essentials for Bacterial growth

Biosynthesis requirements involve an assortment of essential compounds that are essential for the intricate procedures that facilitate bacterial cell division and growth. Key Biosynthetic components are given below.

1. Essential Building Blocks - Necessary for biomolecule production
2. Precursors - Act as metabolic intermediates
3. Cofactors - Support enzyme functions

It includes essential components, precursors and cofactors, which are considered as the building blocks of biomolecule production and guarantee the longevity and effective operation of the microbial cell. Growth factors must be added to culture medium in microbiological cultivation, to promote bacterial multiplication. The restricted availability of essential components provides nutrition resources creating unavailable for bacterial production even in trace levels.⁽¹⁷⁾ Growth factors are essential for enhancing bacterial growth and overall culture performance to correct these inadequacies. Protein synthesis is a process that requires amino acids, which are also growth factors.

Nutrients in culture media

Nutrients are essential for bacterial growth, metabolism, and cellular functions.⁽¹⁸⁾ Culture media provide nutrients in the form of:

1. Carbon sources - Sugars, alcohols
2. Nitrogen compounds - Amino acids, peptones
3. Minerals - Magnesium, calcium, iron
4. Vitamins - Essential for enzymatic activity

Mineral salts act as enzyme cofactors and support metabolic functions, its role are provided in table 4.

Table 4. Role of Minerals in Bacterial Growth	
Mineral	Function
Phosphorus (P)	Component of DNA, RNA, and ATP
Trace metals (molybdenum, manganese, copper, zinc)	Required in enzymatic reactions

Water and Evaporation Concerns in Agar Incubation:

- Water helps with nutrient solubility, transport, and hydrolysis.⁽¹⁹⁾
- Some bacteria require free water for survival.
- Water loss during incubation can reduce colony size and inhibit bacterial growth.
- Maintaining adequate moisture is crucial for successful bacterial culture.

Vitamins

The selective bacterium *Bifidobacterium longum* possesses special requirements for many vitamins as vital growth factors. The mineral folic acid, riboflavin, vitamin B12, and biotin are necessary for the metabolism of enzymes as they act as coenzymes or biochemical precursors. The bacterium requires volatile fatty acids, other growth factors, and vitamins. Collectively, these chemicals sustain the complex metabolic processes that allow *Bifidobacterium longum* to proliferate and remain stable in the gut microbiota. In culture medium, blood and its byproducts are rich sources of nutrients that encourage the development of certain microorganisms. Essential elements including vitamins, minerals, and amino acids found in blood assist bacterial metabolism and reproduction.

Selective culture media

The purpose of selective culture medium is to encourage the development of certain bacteria while preventing the growth of other germs.⁽²⁰⁾ These media include nutrients tailored to the metabolic requirements

of certain bacteria and other components that foster their development while excluding the growth of undesirable organisms using the use of inhibitors or selective agents.⁽²¹⁾ From diverse microbial populations, specific bacterial species were isolated and identified using selective culture medium. Antibiotics are potent medications that interfere with essential cellular functions to prevent or eradicate bacterial development. Antibiotics are manufactured synthetically or derived from microorganisms and are used to treat bacterial illnesses. It has altered medicine. They interfere with the capacity of certain bacteria to live and multiply by targeting their structures or functions. However, improper or excessive use of antibiotics might result in resistance, which is an important issue in health around world.

Chemical constituents

Chemical additives in culture media create a controlled environment that prevents the growth of undesired bacteria. These chemicals are essential for microbial examination, identification, and isolation. Inhibiting drugs such as penicillin and streptomycin are widely used, while erythromycin targets protein synthesis, and tetracycline prevents bacterial replication. In addition, selective agents such as methylene blue or crystal violet hinder bacterial metabolism. Malachite green inhibits Gram-positive bacteria, and acriflavine disrupts bacterial DNA functions.

Commonly used inhibitors include

- Lithium chloride: Used for DNA extraction due to its ability to precipitate DNA. Sodium perchlorate also enhances nucleic acid purity.
- Lauryl sulfates: Surfactants that damage bacterial cell membranes. Cetyltrimethylammonium bromide (CTAB) disrupts bacterial lipid structures.
- Irgasan: Known for its antibacterial properties, while triclosan inhibits bacterial growth by disrupting fatty acid synthesis.

ANTISEPTICS

Antiseptics prevent bacterial growth on living tissues, while disinfectants are used for non-living surfaces. Common antiseptics include alcohol, iodine-based solutions, hydrogen peroxide, and chlorhexidine. Ethanol and isopropyl alcohol are widely used, while benzalkonium chloride disrupts bacterial cell membranes, and povidone-iodine releases free iodine to kill microbes.

Cetrimide is a selective antiseptic used for isolating *Pseudomonas aeruginosa*, while polymyxin B targets Gram-negative bacteria, and sodium lauryl sulfate inhibits bacterial growth by disrupting membranes. Chlorhexidine is an effective antiseptic, while octenidine has broad-spectrum antimicrobial effects.

DYES

Dyes in culture media function as color indicators and selective agents targeting specific microorganisms. Crystal violet inhibits bacterial growth, while:

- Malachite green: Used to inhibit Gram-positive bacteria.
- Brilliant green: Targets Gram-positive species.
- Ethyl violet: Works similarly to crystal violet but with slightly different microbial selectivity.

Methylene blue is used to target Gram-positive bacteria, while phenol red changes color based on bacterial metabolism, helping to distinguish microbial strains

SODIUM SALTS

Sodium salts are used in culture media to create selective conditions for microbial growth. Sodium chloride is commonly employed, while:

- Potassium chloride (KCl): Maintains osmotic balance while influencing bacterial growth.
- Sodium sulfate (Na₂SO₄): Used in selective media to inhibit non-halophilic bacteria.
- Sodium deoxycholate: Damages bacterial membranes, while bile salts serve similar functions.

Sodium azide is commonly used to inhibit Gram-positive bacteria, while potassium tellurite has similar inhibitory effects on certain bacterial strains.

Non-Selective Culture Media

Development media called non-selective culture media are made to encourage the development of various microorganisms without favoring or preventing the growth of certain varieties. These substrates offer an overall nutrient-rich environment that promotes the growth of different microorganisms.

Tryptic Soy Agar (TSA)

In microbiology TSA is a frequently utilized non-selective medium for growth, which provides an environment with full of nutrients conducive to the development of bacteria. TSA is made up of soybean meal and casein enzymatic digests and it is enriched with various nutrients to offer the ideal growth medium. Peptones made from soybean meal and casein is utilized in the formulation as a source of nitrogen and amino acids. The components act as the main sources of nutrition for the development of bacteria.⁽²²⁾ To reinforce the medium and enable a simple culture of microorganisms in a petri dish, agar, a polysaccharide generated by seaweed is added. Bacterial isolation, typical laboratory work, and maintaining bacterial stocks are the areas where it is highly beneficial. It is advantageous to get an accurate portrayal of the microbial population since the medium is non-selective and lacks chemicals that might prevent the development of certain bacteria.

Luria-Bertani (LB) Agar

LB Agar is commonly used for non-selective growth medium in bacterial microbiology. LB Agar which was created first for *Escherichia coli*, has become a mainstay in bacterial analysis. Peptide, yeast extract, sodium chloride, and agar are the usual ingredients of LB Agar. While sodium chloride preserves osmotic equilibrium, peptone and yeast extract to supply vital minerals and amino acids. To enable the growth of distinct colonies, agar solidifies the media.⁽²³⁾ Cloning bacteria and maintaining bacterial cultures are two applications where LB Agar is ideal. Sample collection of bacterial populations is achievable since it is not selective. LB Agar is used in molecular biology procedures such as recombinant protein expression and plasmid amplification.

Plate Count Agar (PCA)

In microbiology, PCA is a typical growth medium that is used to enumerate and enumerate live bacteria present in a given sample. PCA is especially useful in the estimation of the amount of microorganisms present in food, water, and environmental sample samples. The composition of Plate Count Peptone, yeast extract, glucose and agar are generally incorporated in agar. Yeast extract and peptone provide amino acids and minerals, and glucose serves as the energy source for microbial growth. By solidifying the media, agar allows for the growth of individual colonies which are easy to enumerate. Dilution of the sample and the use of a known volume on PCA plates are the plate count technique steps. Every viable microbe replicates to form an apparent colony when incubated. The former can quantify the initial microbial burden in the sample by counting colonies. The method is crucial for research studies of microbial populations, food monitoring, water quality monitoring, and assessment of the effectiveness of antimicrobial treatments.⁽²⁴⁾ PCA is a critical technique in microbiology laboratories due to its reliability and simplicity. It can be helpful to understand microbial dynamics in various cases, and it provides a quantitative measurement of living bacteria.

Fluid Thioglycollate Medium (FTM)

Microbiologists utilize a pliable liquid culture medium, referred to as FTM, for the cultivation and research of growth characteristics of microbes, particularly microbes with fluctuating oxygen needs. FTM has been prepared in a manner to support the growth of anaerobic and aerobic bacteria.⁽²⁵⁾ There are certain constituents of FTM, which play the role of creating an oxygen content gradient of the medium. Sodium thioglycollate, peptones, yeast extract, dextrose, and agar constitute the main constituents of FTM. Dextrose is used as a source of energy, peptones and yeast extract provide essential nutrients. Sodium thioglycollate reduces the oxygen level in the medium, which helps in establishing a low-oxygen environment. Over all, this review explores advancements in clinical microbiology by surpassing traditional boundaries in bacterial culture media, balancing selectivity and inclusivity for improved diagnostics.

CONCLUSION

Bacterial culture techniques are experiencing a renaissance due to the rapid growth of microbiological methodologies, especially the advances in metagenomics. Improvements in customized media that meet specific testing needs, an advanced comprehension of microbial diversity are indicative of progress. Water and vital nutrients are needed for bacterial development in culture medium. Phenotypic and culture-based methods continue to be essential for detecting and characterizing a wide range of pathogens in clinical microbiology, even in the face of major advancements in testing techniques. Molecular biology techniques and microscopy are the examples of empirical approaches which are essential to solve the riddles of the microbial world. Microorganisms are cultivated and examined for a variety of uses in analysis, industry, and clinical diagnostics applying both non-selective media like Tryptic Soy Agar and enhanced selective culture media. Future developments in metagenomics and other cutting-edge technologies will allow more customization and improvement of bacterial culture medium in clinical microbiology. This will improve the media's capacity to identify a wide range of efficiency and specificity of pathogens.

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