Assessment of Culture Media in Pharmaceutical Microbiology

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Introduction

Culture media is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible, and repeatable microbiological test results is reduced [1]. A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms. Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents (for solid media) [2]. Culture media has been used by microbiologists since the nineteenth century. Even with the increased use of rapid methods the majority of techniques found in the pharmaceutical quality control laboratory require growth media [3]. For the assessment of culture media, no one definitive standard exists. In light of this, this article presents some considerations for designing the testing regime and for the selection and control of microorganisms.

Types of Culture Media

There is a range of different culture media available. Different types of culture media are typically divided, based on the physical state of the media, into:

- Liquid culture media, commonly called "broth"
- Solid and semi-solid culture media, commonly called "agar"

Such media can then be further divided into such categories as growth media (designed to grow most heterotrophic microorganisms), transport media (for preserving microorganisms), enrichment media (media designed to increase the numbers of desired microorganisms), and selective growth media.

The pharmaceutical microbiology laboratory uses a range of culture media depending upon the application required [4]. The two common general medium types are nutrient agar or broth, and tryptone soya agar or broth. Tryptone soya agar (equivalent to soyabean casein digest medium), in particular, is widely used for environmental monitoring. This medium is used for the isolation and cultivation of non-fastidious and fastidious micro-organisms [5]. Tryptone soya broth is used for sterility testing and as a general growth broth in microbial enumeration tests, as well as used for media simulation trials [6]. For some media filling trials, vegetable peptone broth is used as a replacement as it contains no animal products.

Other media types used include fluid thioglycollate medium, used for the growth of bacteria (aerobic and anaerobic) as part of the sterility test. Where monitoring for fungi is required, such as part of an environmental monitoring regime, the commonly used media are sabouraud dextrose agar or malt extra agar. For the microbiological examination of water, R2A is used. This is a low nutrient agar used for the cultivation of heterotrophic microorganisms. Other media are used for microbiological identification, such as Columbia blood agar (for the detection of hemolytic reactions by Staphylococci).

The manufacture of media is done either in-house (whereby a dehydrated formulation is used) or, more commonly, is purchased readymade [7]. Where media is purchased ready-to-use, the microbiologist has a responsibility to audit the manufacturer of the media [8]. For certain plate media, such as that used in cleanrooms, the media should be sterilized by irradiation [9].

Quality Control of Culture Media

It is important that each batch of such media undergoes some form of quality control before it is released for general use to provide a measure of confidence that the results issued from microbiology laboratories are accurate. Testing is normally undertaken once all preparatory steps have been completed, including irradiation.

The quality control of culture media can be divided into two parts:

Physical Characteristics

The tests undertaken for the physical characteristics of culture media vary depending upon the type of media. Examples of physical tests include:

- Visual test for color: The color of a sterilized medium should be compared to a non-sterilized medium and any differences in color noted.
- Visual test for clarity: The clarity of the media should be examined for optical artefacts, such as crystallization.
- Gel strength: The gel strength should not be over-hard or over-soft, but firm and usable.
- pH of the finished media: This is probably the most important chemical test, because if a pH is outside of the recommended range for the media, then this will lead to the inhibition of some of the microorganisms that the media is intended to grow [10].
- Checks for damage: Plates and bottles should be examined for damage like cracks and defects.

Microbiological Characteristics

1. The test of media sterility is designed to detect microbial contamination during the manufacturing process. Here a small number, normally 2% of the batch, of uninoculated items are incubated. The temperature and time selected for the sterility test incubation will depend upon the type of media. For general-purpose media, a temperature of 30-35°C for three days is typical. To pass the sterility test the items must demonstrate no growth.
2. Arguably the challenge of culture media with microorganisms is the most important test carried out in the microbiology laboratory. That such a key test is undertaken by the media manufacturer is unquestionable. Additionally it is common for the purchaser to carry out growth promotion, check for batch-to-batch variability, or assess any issues during shipment [11].

For growth promotion a panel of microorganisms is required to demonstrate the suitability of the media for its intended use. The pharmacopeia recommends certain microorganisms and these must be traceable to a reputable culture collection, such as the American Type Culture Collection (ATCC) (although the pharmacopeia allows for alternative culture collections to be used there is some ambiguity about strain equivalency). Type cultures should be carefully preserved within the culture collection of the laboratory. This includes ensuring that cultures are held at a temperature low enough to avoid phenotypic variations from occurring and restricting the number of passages between subculture steps to less than five [12].

The standard set of typed cultures detailed in the European Pharmacopeia and United States Pharmacopeia are shown in *Table 1*.

Table 1. Standard Media Growth promotion Test Microorganisms

Microorganism	Culture Collection Reference
Staphylococcus aureus subsp. aureus	ATCC 6538
Bacillus subtilis subsp. spizizenii	ATCC 6633
Pseudomonas aeruginosa	ATCC 9027
Clostridium sporogenes	ATCC 19404
Candida albicans	ATCC 10231
Aspergillus brasilensis	ATCC 16404
Escherichia coli	ATCC 8739
Salmonella enterica subsp. enterica serovar *typhimurium*	ATCC 13311

These microorganisms have been serially sub-cultured in national culture collections over decades and are conditioned for growth on rich laboratory culture media. They are designed to allow the vendor to assess the media as suitable at the point of manufacture and for the user to verify the media upon receipt.

In addition to type cultures, environmental isolates are commonly used in media testing regimes. These organisms are designed to demonstrate that a particular lot of culture media will grow microorganisms which are representative of the types that are found in the manufacturing environment [13]. Thus media used for the examination of water would have a test panel which included microbial isolates from water (such as Pseudomonad related bacteria) and media used for environmental monitoring would include bacteria transient to human skin (such as Staphylococci).

While the use of such isolates is increasingly becoming a regulatory expectation, the adoption of environmental or plant isolates is not supported by all microbiologists. Arguments for the use of such isolates are that the media is challenged with those microorganisms actually encountered within the pharmaceutical environment, and that these are often more representative than the standard cultures. Moreover, the isolates can be varied over time, based on reviews of microflora, so that they remain so perpetually relevant. Arguments against include the fact that inter-laboratory assessments are rendered difficult because each laboratory is using a different organism set. A second point is that once organisms are grown on standard media they become indistinguishable from other laboratory strains. It has been counter-argued that minimally sub-cultured environmental isolates have aspects of their "wild type" attributes conserved. The outcome of this debate is ongoing and clearly further study is needed.

Test Methods and Acceptance Criteria

The numerical level of the microbial challenge is another important consideration. Most testing regimes require a low level challenge. This is to show that the media can recover low numbers of microorganisms. In most this is a challenge of <100 microorganisms [14].

Solid Media

There are various qualitative and quantitative approaches that can be taken for the testing regime. For the testing of agar, qualitative approaches include simple sub-culture streaks (spread plates). Here, liquid cultures are streaked with an inoculation loop to give single colonies. Each segment of the agar plate can then be compared to the growth characteristics of a suitable control plate (a control medium is a released batch of media which has previously been assessed as having good growth promotion properties). A more robust system is ensured through quantitative techniques. These generally fall into two groups: the ecometric and the Miles-Misra [15]. Both of these tests compare one set of media (a previously released batch) against another (the batch to be tested). The ecometric method is a semi-quantitative variant of the streaking method [16]. One loopful of inoculm is placed onto the plate and is sequentially diluted streak to streak. Five streaks are streaked out into four quadrants onto the agar plate along with a final streak in the center of the plate. Growth should occur in all streaks.

The Miles-Misra technique (the drop count technique) involves spreading droplets of known quantities of microbial suspensions (typically 10 µL). The test plate is compared to a control plate, after incubation, in terms of the number of colonies recovered. The accuracy of the method is dependent upon the dilution used, the number of colony forming units in the inoculum, the volume of the inoculums used, and the spreading technique [17]. The result is typically

expressed as a productivity ratio when, after incubation, the count of a previously released batch of media is divided into the count of the test media [18]. An acceptable productivity ratio must be equal to or greater than 0.5 and with an upper limit of 2.0 (this is equivalent to a 50-200% recovery).

Broth Media

For broth (liquid) media it is less easy to apply a quantitative assessment. Many laboratories challenge broth media with an estimated number of microorganisms and compare the growth, over time, with a control batch (which provides a qualitative assessment of copious growth). The challenge is typically <100 cfu and the time to obtain growth is between three and five days. The growth between the test batch and the control batch is then compared with the requirement that both must show copious growth. Alternatively, some laboratories attempt a semi-quantitative approach by constructing a growth index from slight to copious growth (normally a scale of +, ++, or +++).

Test Regime

Once decisions relating to the type of microorganisms and the test method have been made, the question of the test conditions arises. Many laboratories use general media which may be used at a range of temperatures, yet to test this media at every temperature that it could potentially be used at could be expensive and could create an unwieldy release system. A practical approach is to test at the midrange temperature. However, any regime will need to be defensible to regulatory authorities.

Incubation time is another parameter that requires careful planning. For some media this is clearly defined in the pharmacopoeias (typically growth of bacteria within three days and growth of fungi within five days). However, for other media a realistic time must be established based on the application of the media and the types of microorganisms which are used for the challenge.

Expiry Time Assessment of Culture Media

Culture media will have defined storage conditions and an expiry time and the shelf-life needs to be validated [19]. This is to assess if different humidity levels (which can affect the water activity of solid media), chemo-oxidation (due to physical factors like heat), and photo-oxidation (from sunlight) affect the media [20].

Media Release and Quarantine

A media quality control system will need to consider the release criteria and quarantine system. With regard to the release criteria, the laboratory must put in place clear guidelines for the repeat test procedure. This will need to cover invalid tests and the procedure to be followed should any microorganisms fail to grow or show recovery at the expected level [21]. A quarantine system is important in order to prevent media which has not been assessed from entering general use.

Summary

Microbiological culture media is the most widely used and arguably most important tool of the pharmaceutical microbiologist. Given this primacy it is important that the media manufactured or purchased by the laboratory is of high quality and suitable for the intended test method. This article has set out to show that the control and release of microbiological culture media requires a well-thought-out structure.

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Author biography

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