Microbiological culture media

5

5.1 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. Culture media remains important even with the advances with rapid microbiological methods (indeed many rapid methods continue to rely on the growth of microorganisms on a medium). Without high-quality media then 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. Essentially, it is a substance designed to create nutritional conditions similar to the natural environment in which the microorganism commonly survives and reproduces. 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. For the assessment of culture media, no one definitive standard exists [3]. In light of this, this chapter presents some considerations for designing the testing regime and for the selection and control of microorganisms.

5.2 Cultivation

The cultivation of microorganisms on culture media is dependent upon a number of important factors including an optimal array of nutrients, oxygen or other gases, moisture, pH, and temperature. Important nutrients include sources of carbon, nitrogen, inorganic phosphates and sulfur, trace metals, water, and vitamins. Each nutrient is, in varying combinations, a key ingredient of microbiological culture media [4]. The nutrients function as "growth factors." A growth factor is a naturally occurring substance, like an amino acid, which is capable of stimulating cellular growth, proliferation, and cellular differentiation.

5.3 A short history of culture media

The origins of microbiological culture media can be traced to the nineteenth century when the science of bacteriology was just beginning. During this pioneering time, bacteriologists attempted, with variable success, to grow microorganisms either directly using the food or material on which the microorganism had first been observed or some compound thereof. These were primarily beef-based broths of unknown and

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variable composition [5]. Arguably, the first to cultivate microorganisms on a growth medium, with a degree of reproducibility, was the French chemist and microbiologist Louis Pasteur (1922–1985). While acting as the administrator and director of scientific studies at the École Normale (Paris), Pasteur fashioned a media of yeast, ash, candy sugar, and ammonium salts in 1860 [6]. The object was to produce a fermentation medium. This medium contained the basic requirements for microbial growth: nitrogen (ammonium salts), carbon (sugar), and vitamins (ash). In developing the media, Pasteur made some important observations: that particular chemical features of the medium can promote or impede the development of any one microorganism and that competition occurs among different microorganisms for the nutrients contained in the media, which can lead to some species outgrowing and dominating a culture.

A wider application of materials was utilized, and consequently greater success observed, when Robert Koch (1843–1910) discovered that broths based on fresh beef serum or meat extracts (so-called bouillons, the term "broth" for liquid culture medium being analogous to broth or soup) produced optimal growth [7]. Indeed, Koch's work was so groundbreaking that the cognomen "The Father of Culture Media," oft stated in many microbiological textbooks, is not misplaced.

A significant development on from the liquid medium was with solid media. In 1881, Koch demonstrated a new technique at the International Medical Congress in London, at which Pasteur is alleged to acknowledge "C'est un grand progres" [8]. Koch had recognized the difficulties of using broth media for isolation of pure cultures and had looked for solid media alternatives (this inquiry was instrumental in Koch isolating *Bacillus anthracis*, the causative agent of anthrax) for the first time, in 1882, which represented a major step-forward in disease control [9]. Initially Koch evaluated media such as coagulated egg albumen, starch paste, and an aseptically cut slice of a potato. After limited, but ultimately encouraging results, Koch developed a meat extract with added gelatine (a colorless substance derived from the collagen inside the skin and bones of animals). The resulting "nutrient gelatine" was poured onto flat glass plates, which were then inoculated and placed under a bell jar. This new plate technique could be used both to isolate pure cultures of bacteria and to subculture them either onto fresh plates or nutrient gelatine slopes in cotton-wool plugged tubes [10].

Although nutrient gelatine was a major advance, gelatine has disadvantages as a gelling agent. However, 1 year later, Koch's attempts at a nutrient medium were advanced. In 1882, Fannie Eilshemius (née Hesse) (1850–1934) suggested replacing gelatine with agar [11]. Eilshemius had been inspired by the use of agar to prepare fruit jams and jellies (agar had been used as a gelling agent in parts of Asia for centuries. Agar (or "agar–agar") is a phycocolloid water-soluble polysaccharide derived from red–purple seaweeds (the various species of *Rhodophyceae* belonging to the genus Gelidium and Gracilaria). Agar proved to be a superior gelling agent. It is prepared by treating algae with boiling water. The extract is filtered while hot, concentrated, and then dried. Agar has physical properties that could be readily adapted for bacteriology. Agar melts when heated to around 85 °C, and yet when cooled it does not form a gel until it reaches 34–42 °C (a physical property called hysteresis). Agar is also clearer than gelatine, and it resists digestion by bacterial enzymes. The use of agar allows the creation of a medium that can be inoculated at 40 °C in its cooled molten state and yet incubated up

60 °C without melting (a useful characteristic when examining for thermopile bacteria). Typically, a 1–12% final concentration of agar is used for solidifying culture media [12].

The ability to grow bacteria on solid media was to prove a major milestone in the development of bacteriology (and agar went on to have wider application in electrophoresis, as agarose gels, and diffusion assays). The formation of bacteria on solid media led Koch to use the word "colony" to describe the pure and discrete growth [13].

A further important development for the manufacture of solid media occurred in 1887 when Julius Richard Petri (1852–1921), another worker in Koch's laboratory, was involved in modifying the flat glass plate, common to laboratories, and produced a new type of culture dish for media. This was the Petri dish. Petri used a shallow, circular glass dish with a loose-fitting cover to culture bacteria and other microorganisms, by adding gelatine-based culture media into the dish. The key design feature of the Petri dish was the use of an overhanging lid, which was in place to keep contaminants out [14]. While there is some dispute concerning whether Petri invented the "Petri dish," or whether it was in act invented earlier by Emanuel Klein, a Slovenian scientist working in England, its application in the history of microbiology is of great importance. For many years, glass dishes were used, mainly until the mid-1960s, where advances with injection moulding technology led to Petri dishes being manufactured out of clear polystyrene plastic.

Agar provides the structure for solid microbiological media, but it does not provide the nutrients necessary for bacteria to grow. For this, "growth factors" are required (essential substances that the organism is unable to synthesize from available nutrients). With the initial production of culture media, the primary nutrient sources were derived from meat (as noted by Klebs in 1871 and Nageli in 1880 [15], who were the first to record that bacteria grow well in culture media containing partially digested meat proteins or "peptones." Peptones provide a soluble and assimilable form of all the essential mineral contents of living material as well as the organic carbon and nitrogen sources). Although the meat extract used for the earliest culture media was a rich source of many of the necessary growth factors for bacteria, it was insufficient in amino-nitrogen to allow optimal growth of a range microorganisms. In 1884, Fredrick Loeffler added peptone and salt to Koch's basic meat extract formulation. The peptone he used was an enzymatic digest of meat, at the time produced in the nineteenth century as a pharmaceutical product and prescribed for nutritional disorders. This peptone added amino-nitrogen, while the salt raised the osmolarity of the medium [16].

By the 1890s, culture media had developed to form familiar to microbiologist of the twenty-first century: clearer broths; solid media in Petri dishes; and the widespread use of peptones and agar. Nonetheless, it became increasingly apparent that there was a gap in market place for mass-produced culture media. The development of commercially produced culture media originated with the meat industry, whereby hitherto discarded by-products from the manufacture of meat products were used to produce culture media. Arguably, the most prominent example of this was the German Baron Justus von Liebig who, in the nineteenth century, established a reputation as one of the fathers of modern chemistry making important contributions to research and discovery in agriculture, animal chemistry, pharmacology, and food chemistry. The Liebig Extract of Meat Company (LEMCO) was formed in 1865 to manufacture and sell

Liebig's extract of meat. The main source of meat was from the company Fray Bentos in Uruguay (where cattle were slaughtered mainly for their hides and the meat was a little-used by-product) [17].

Liebig developed his meat extract as a food source. His initial motivation was to a provide food source, stable at room temperature, for the growing malnourished poor people in central Europe (a "beef-tea" that he described as his "extraction carnis"). This became the company's most famous product: the Oxo cube. It was only later that a use for the waste product from the manufacture of Oxo cubes was found: to manufacture microbiological culture media. By 1924, the OXO Medical Division of LEMCO and the products were sold to hospitals and laboratories. The ability of OXO to provide media on a large scale was accelerated by the development of dehydrated culture media, whereby media were preserved for long periods by removing water. Low amounts of water resulted in the media powder having a low water activity that reduced the possibility of spoilage occurring. In the United States, similar movements toward mass production occurred during the war undertaken by the American Agar Company of San Diego, California, and by the Digestive Ferments Company (Difco) [18].

Since the early days of Victorian development, culture media has undergone steady development over the past 100 years. Despite better production techniques, lower contamination rates, and improved purity, the basic principles of preparing broths and agars remain the same, and the legacy of the founding parents of bacteriology continues to be of the utmost importance.

5.4 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:

(a) liquid culture media, commonly called "broth" (Figure 5.1);
(b) solid and semi-solid culture media, commonly called "agar" (Figure 5.2).

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 [19]. 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 nonfastidious and fastidious microorganisms [20]. 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 [21]. 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 a part of the sterility test. Where monitoring for fungi

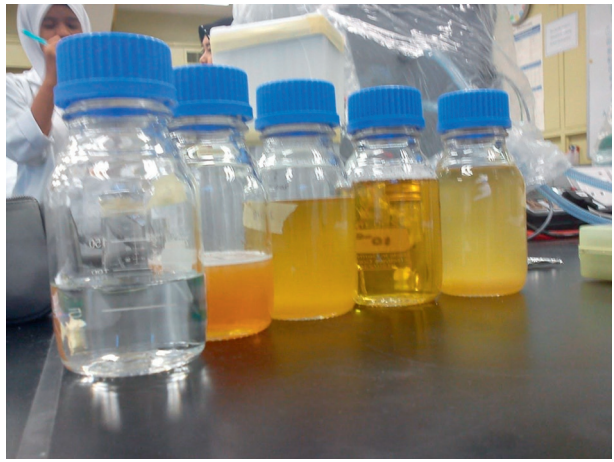

Figure 5.1 Microbiological broth media, some exhibiting turbidity.
Photograph: Tim Sandle.

Figure 5.2 A microbiological agar plate, showing a streak plate method to obtain single colonies.
Photograph: Tim Sandle.

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 either manufactured "in-house" (whereby a dehydrated formulation is used) or, more commonly, purchased ready-made [22]. Where media is purchased ready-to-use, the microbiologist has a responsibility to audit the manufacturer of the media [23]. For certain plate media, such as those used in cleanrooms, the media should be sterilized by irradiation [24].

5.5 Quality control of culture media

The European and US pharmacopoeias describe the application of culture media for several tests, primarily sterility, microbial enumeration, and the examination of pharmaceutical grade water [3]. There is, however, no excepted international standard for the testing of culture media applicable to the pharmaceutical industry. There is a standard, divided into two parts, written for the food industry: ISO11133 [25,26] but one that is often drawn upon by pharmaceutical microbiology. Part one is concerned with the general terminology related to quality assurance and specifies the minimum requirements for the preparation of culture media to be used for the microbiological analysis; and part two relates to the criteria and methods for the performance testing of culture media. The standard is of further importance given that aspects of it have formed the basis of accepted testing regimes recommended by culture media manufacturers.

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 and microbial characteristics.

5.5.1 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 with 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, for 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 [27];
- Checks for damage: plates and bottles should be examined for damage like cracks and defects.

5.5.2 Microbiological characteristics

(a) 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 3 days is typical. To pass the sterility test, the items must demonstrate no growth.

(b) Arguably the challenging 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, to check for batch-to-batch variability or to assess any issues during shipment [28].

For growth promotion, a panel of microorganisms is required to demonstrate the suitability of the media for its intended use. Where the pharmacopeia recommends certain microorganisms and that 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 fewer than five [29].

The standard set of typed cultures detailed in the European Pharmacopeia and United States Pharmacopeia are shown in Table 5.1.

These microorganisms have been serially subcultured in national culture collections over decades and are conditioned to 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 that are representative of the types that are found in the manufacturing environment [30]. Thus, media used for the examination of water would have a test panel that included microbial isolates from water (such as

Table 5.1 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

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 are 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 interlaboratory 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 subcultured environmental isolates have aspects of their "wildtype" attributes conserved. The outcome of this debate is ongoing, and clearly further study is needed.

5.5.3 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 fewer than 100 microorganisms [31].

5.5.4 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 subculture 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 [32]. 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 semiquantitative variant of the streaking method [33]. 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 with 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 (cfu) in the inoculum, the volume of the inoculums used, and the spreading technique [34]. 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 [35]. This is calculated using the following formula, based on the two duplicate samples for the test plate and control plate:

$$\text{Productivity ratio} = \frac{\text{Mean of two test plates}(\text{cfu})}{\text{Mean of two comparative control plates}(\text{cfu})}$$

For example:

Test plates:	32 and 40 cfu (mean 36 cfu)
Control plates:	50 and 46 cfu (mean 48 cfu)
Productivity ratio:	36/48 = 0.8

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).

5.5.5 Broth media

For broth (liquid) media, it is more difficult 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 fewer than 100 cfu, and the time to obtain growth is between 3 and 5 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 semiquantitative approach by constructing a growth index from slight to copious growth (normally a scale of +, ++, or +++).

5.5.6 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 that may be used at a range of temperatures, yet to test this media at every temperature that it could potentially be used could be expensive and could create an unwieldy release system. A practical approach is to test at the mid-range 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 5 days). However, for other media, a realistic time must be established based on the application of the media and the types of microorganisms that are used for the challenge.

5.5.7 Testing of selective media

Selective media requires a slightly different approach in that the aim is not only to see if the media supports the growth of a range of microorganisms, but also to examine inhibition and colony morphology and pigment. Here positive and negative control

strains are required. In order to ascertain growth characteristics for selective media, some microorganisms are used as positive or negative indicators of growth. For the positive reaction, particular colony morphologies, pigmentation, or diffusion of activity may be part of the acceptance criteria.

5.5.8 Expiry time assessment of culture media

Culture media will have defined storage conditions and expiry time and the shelf-life needs to be validated [36]. This is to assess if different humidity levels (which can affect the water activity of solid media), chemo-oxidation (due to physical factors such as heat) and photo-oxidation (from sunlight) affect the media [37].

5.6 Manufacture of culture media

The manufacture of media is an important process for a microbiology laboratory. The manufacture of media either manufactured "in-house" (within the microbiology laboratory of the pharmaceutical company whereby a dehydrated formulation is used) or, more commonly, purchased from the manufacturer ready-made or from a contract manufacturing laboratory (this media can be for immediate use, such as agar in a Petri dish or media that is partially completed and requires an interim preparatory step prior to use, such as melting). Even where media are purchased ready-to-use, the microbiologist has a responsibility to audit the manufacturer of the media and to have an understanding of the media manufacturing process.

In setting up a media preparation area (sometimes referred to, especially in North America, as the media kitchen), it is important for the microbiologist to build in quality to the design of the workflow and the key manufacturing steps. Important manufacturing concepts, such as batch rotation, for example, should apply to the use of media powder. As with other pharmaceutical manufacturing processes, aseptic techniques should be adopted at all times to minimize the opportunity for the media to become contaminated.

The key stages for media manufacture are [23]: initial preparation, rehydration, sterilization, addition of supplements, filling, labeling, and secondary sterilization.

5.6.1 Initial preparation

Prior to manufacturing, the types of media and quantities required should be planned out. When complex media are required purchasing, a premixed powder is the most straightforward option (powdered media in this form is supplied by several different companies). Ready-to-use powder is not available for all types of media, and with such cases, various individual constituents will need to be purchased.

Consideration should be given to the appropriate vessel for holding or dispensing the media. For the preparation of broth, it is normal to dispense the broth into the required in-use containers (commonly glass bottles) prior to sterilization. The use of an accurate dispensing device is required for this. A regular check of the volume

dispensed, by measuring volume or more commonly by verifying the weight, is an important quality control step. For plate media, it is more common for the media preparation to be sterilized, then cooled, and then dispensed into Petri dishes. The fill volume of the plates should be confirmed during dispensing.

Some laboratories elect to perform microbiological testing on the received dehydrate powder (where there is a particular risk from spore-forming microorganisms that might, in sufficiently high numbers, survive the sterilization step). This is a separate activity to the more universal postmanufacture quality control testing of the prepared culture media.

5.6.2 Rehydration

The preparation of a culture medium requires the components to be dissolved. Media powder is rehydrated by mixing a measured amount of the medium in the required volume of water. The water should be freshly prepared and, depending on the type of media, held at a warm temperature. Instructions for rehydration are usually printed on the container (e.g., 30 g/L for agar X into 5 L of water). When undertaking this step, it is important to maintain the homogeneity of the solution by mixing. With media containing agar as a solidifying agent, the media are hydrated by gently heating and agitating the water: media mixture to dissolve it. Care must be taken to avoid scorching the media. Here, the media should clarify near boiling (95–100 °C), and the media should only be allowed to boil for a brief period of time (less than 1 min).

5.6.3 Sterilization

The sterilization of the media plays an important role in the quality of the media. Most incoming powdered media contain a level of contamination, and if the media is incorrectly sterilized (to eliminate viable microorganisms), then spoilage could develop thereby rendering the media unusable. Most media are sterilized by steam under pressure (autoclaving) or, for some plate media, in an agar preparator. For some special culture, media sterilization is performed by filtration (where heat would destroy one or more growth factors). For all media and reagents that are sterilized by autoclaving, a sterilization batch number should be assigned. The procedure for assigning the sterilization batch number should follow a simple and similar notation. A suitably qualified autoclave should be used.

For containers holding agar media prior to dispensing, the container should be held at around 50 °C in preparation for filling. The medium should be dispensed as soon as it equilibrates to 45–50 °C or within a maximum time (3 h is a typical target time). It is recommended that the medium is mixed gently prior to dispensing.

5.6.4 Addition of supplements

For some types of media, additives or supplements are required. Most of these components cannot be sterilized by autoclaving as they are heat labile, such as buffers and amino acids. For these materials, the standard practice is to sterilize them by

membrane filtration (the removal of microorganisms from the liquid). The type of filter used will depend upon the solution type. Care must be taken when selecting the material of the filter to avoid any leechables contaminating the filtrate. Such supplements need to be added to the media after sterilization, when the media are held at a cooler temperature in the water bath.

5.6.5 Filling

For agar, the sterilized media require dispensing into Petri dishes. This activity requires utmost caution to avoid contamination. To prepare filled Petri dishes, the sterilized media should be carefully tempered while in the molten state (to around 45–50 °C, gelling typically occurs between 32 and 40 °C) and then dispensed into sterile, glass, or plastic Petri dishes. Petri dishes are typically of a 95–100 mm diameter or to a 50–55 mm diameter (for contact plates used for surface sampling as parts of an environmental monitoring regime) and are purchased in sleeves of dishes (often with 20 or 50 plates per sleeve). Agar does not normally distribute uniformly when melted and requires mixing to ensure a uniform distribution. The filling activity is normally a semiautomated process using dish fillers. Periodic assessments of the volume filled must take place during the filling operation. This check is normally made by weighing a selection of filled plates. The dispensing of plates should take place under a unidirectional airflow hood to minimize contamination. This is necessary to avoid contamination even if the plates are to be subject to a secondary sterilization step such as irradiation.

5.6.6 Labeling

Once the sterilization and filling steps have been competed, all sterilized broth bottles and filled plates must be labeled, and the details recorded in the batch record. Labels should specify the media type, batch number, expiry time, and storage conditions.

5.6.7 Secondary sterilization

For certain plate media, such as that used in cleanrooms, an additional sterilization step can be undertaken. The most common method for this is irradiation by ionization using gamma rays. The effectiveness of the irradiation should also be checked by a sterility test of the media (which is discussed below). The sterility check is important given that the application of the irradiation dose and the combination of different loading patterns can affect the process [20].

5.7 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 [38]. A quarantine system is important in order to prevent media that have not been assessed from entering general use.

5.8 Summary

Microbiological culture media are 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 are of high quality and suitable for the intended test method. This chapter has set out to show that the control and release of microbiological culture media require a well thought-out structure. The chapter has also considered some of the important practices to observe in relation to the manufacture of culture media. Given the importance of culture media in relation to different microbiologists' tests, the importance that should be paid to media manufacture, control, testing and release cannot be underestimated: culture media is the bedrock of most microbiological examinations.

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