Sridhar Rao PN
Assistant Professor
Dept. of Microbiology
JJMMC, Davangere
www.microrao.com

Bacterial culture media

One of the most important reasons for culturing bacteria in vitro is its utility in diagnosing infectious diseases. Isolating a bacterium from sites in body normally known to be sterile is an indication of its role in the disease process. Culturing bacteria is also the initial step in studying its morphology and its identification. Bacteria have to be cultured in order to obtain antigens from developing serological assays or vaccines. Certain genetic studies and manipulations of the cells also need that bacteria be cultured in vitro. Culturing bacteria also provide a reliable way estimating their numbers (viable count). Culturing on solid media is another convenient way of separating bacteria in mixtures.

History:

Louis Pasteur used simple broths made up of urine or meat extracts. Robert Koch realized the importance of solid media and used potato pieces to grow bacteria. It was on the suggestion of Fannie Eilshemius, wife of Walther Hesse (who was an assistant to Robert Koch) that agar was used to solidify culture media. Before the use of agar, attempts were made to use gelatin as solidifying agent. Gelatin had some inherent problems; it existed as liquid at normal incubating temperatures (35-37^{o}C) and was digested by certain bacteria.

Composition of culture media:

Bacteria infecting humans (commensals or pathogens) are chemoorganoheterotrophs. When culturing bacteria, it is very important to provide similar environmental and nutritional conditions that exist in its natural habitat. Hence, an artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat. Most often, a culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors. Besides these, the pH of the medium must be set accordingly. Some of the ingredients of culture media include water, agar, peptone, casein hydrolysate, meat extract, yeast extract and malt extract.

Classification:

Bacterial culture media can be classified in at least three ways; Based on consistency, based on nutritional component and based on its functional use.

1) Classification based on consistency:

Culture media are liquid, semi-solid or solid and biphasic.

A) Liquid media: These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths” (e.g nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity. Certain aerobic bacteria and those containing fimbriae (Vibrio & Bacillus) are known to grow as a thin film called ‘surface pellicle’ on the surface of undisturbed broth. *Bacillus anthracis* is known to produce stalactite growth on ghee containing broth. Sometimes the initial turbidity may be followed by clearing due to autolysis, which is seen

in penumococci. Long chains of Streptococci when grown in liquid media tend to entangle and settle to the bottom forming granular deposits. Liquid media tend to be used when a large number of bacteria have to be grown. These are suitable to grow bacteria when the numbers in the inoculum is suspected to be low. Inoculating in the liquid medium also helps to dilute any inhibitors of bacterial growth. This is the practical approach in blood cultures. Culturing in liquid medium can be used to obtain viable count (dilution methods). Properties of bacteria are not visible in liquid media and presence of more than one type of bacteria can not be detected.

B) Solid media: Any liquid medium can be rendered by the addition of certain solidifying agents. Agar agar (simply called agar) is the most commonly used solidifying agent. It is an unbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera Gelidium. Agar is composed of two long-chain polysaccharides (70% agarose and 30% agarapectin). It melts at 95^{o}C (sol) and solidifies at 42^{o}C (gel), doesn't contribute any nutritive property, it is not hydrolyzed by most bacteria and is usually free from growth promoting or growth retarding substances. However, it may be a source of calcium & organic ions. Most commonly, it is used at concentration of 1-3% to make a solid agar medium. New Zealand agar has more gelling capacity than the Japanese agar. Agar is available as fibres (shreds) or as powders.

C) Semi-solid agar: Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains (U-tube and Cragie's tube). Certain transport media such as Stuart's and Amies media are semi-solid in consistency. Hugh & Leifson's oxidation fermentation test medium as well as mannitol motility medium are also semi-solid.

D) Biphasic media: Sometimes, a culture system comprises of both liquid and solid medium in the same bottle. This is known as biphasic medium (Castaneda system for blood culture). The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply tilted to allow the liquid to flow over the solid medium. This obviates the need for frequent opening of the culture bottle to subculture.

Besides agar, egg yolk and serum too can be used to solidify culture media. While serum and egg yolk are normally liquid, they can be rendered solid by coagulation using heat. Serum containing medium such as Loeffler's serum slope and egg containing media such as Lowenstein Jensen medium and Dorset egg medium are solidified as well as disinfected by a process of inspissation.

2) Classification based on nutritional component:

Media can be classified as simple, complex and synthetic (or defined). While most of the nutritional components are constant across various media, some bacteria need extra nutrients. Those bacteria that are able to grow with minimal requirements are said to non-fastidious and those that require extra nutrients are said to be fastidious. Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria. Complex media such as blood agar have ingredients whose exact components are difficult to estimate. Synthetic or defined media such as Davis & Mingioli medium are specially prepared media for research purposes where the composition of every component is well known.

3) Classification based on functional use or application:

These include basal media, enriched media, selective/enrichment media, indicator/differential media, transport media and holding media.

A) Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal medium.

B) Enriched media: Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally

exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media.

C) Selective and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

D) Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water are used to recover pathogens from fecal specimens.

E) Differential media or indicator media: Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Exmples: MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

F) Transport media: Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Cary Blair medium and Venkatraman Ramakrishnan medium are used to transport feces from suspected cholera patients. Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery.

G) Anaerobic media: Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Robertson cooked meat that is commonly used to grow Clostridium spps medium contain a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin. Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the thioglycollate medium. Under reduced condition, methylene blue is colourless.

Preparation and preservation

Care must be taken to adjust the pH of the medium before autoclaving. Various pH indicators that are in use include phenol red, neutral red, bromothymol blue, bromocresol purple etc. Dehydrated media are commercially available and must be reconstituted as per manufacturers' recommendation. Most culture media are sterililized by autoclaving. Certain media that contain heat labile components like glucose, antibiotics, urea, serum, blood are not autoclaved. These components are filtered and may be added separately after the medium is autoclaved. Certain highly selective media such as Wilson and Blair's medium and TCBS agar need not be sterilized. It is imperative that a representation from each lot be tested for performance and contamination before use. Once prepared, media may be held at 4-5^{o}C in the refrigerator for 1-2 weeks. Certain liquid media in screw capped bottles or tubes or cotton plugged can be held at room temperature for weeks.