Applied Veterinary Bacteriology and Mycology: Bacteriological techniques
Chapter 2: Preparation and use of culture media

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GENERAL CONDITIONS PERTAINING TO THE CULTURE OF MICRO-ORGANISMS

To study bacteria, their characteristics and relationships to animals and man, it is necessary to be able to cultivate them in a laboratory.

The growth of micro-organisms on or in culture media is dependent on a number of very important factors:

- The required nutritional elements must be available.
- The correct environmental atmosphere must be provided.
- A certain degree of moisture is required.
- The medium must have the proper pH value and salt concentration.
- Ideal temperature conditions must prevail.
- The isolation medium must be sterile.
- Contamination must be prevented.

A satisfactory microbiological medium must contain available sources of carbohydrates, amino acids, inorganic salts and, in certain cases, vitamins or other growth promoting substances. These were originally supplied in the form of meat infusions and later beef extracts. Although these are still used today, their preparation causes the loss of heat-labile nutrients. Peptones are more commonly used nowadays, because they supply a ready source of nitrogen in the form of amino acids and simple nitrogenous compounds as well as carbon. Certain bacteria require additions of other substances such as serum or blood to the growth medium. Carbohydrates may also be desirable at times, and certain elements such as calcium, manganese, magnesium, sodium and potassium may be required. Vitamins may be required to promote the growth of certain bacteria. An example is vitamin K which promotes the growth of Prevotella melaninogenica. Dyes may be added to media as indicators of metabolic activity or because of their selective inhibitory powers.

Agar is used as a solidifying agent in certain media. It is made from seaweed (Gelidium corneum) and consists of non-nutritive carbohydrates. Agar melts at 95 - 98°C and solidifies at 40 - 42°C. It is usually added at 1 - 1.5% to produce solid media and at 0.1% - 0.3% to produce semi-solid media.

A wide variety of media formulations are available for use in the culture of bacteria and fungi. They may be fluid, semi-solid or solid. Liquid media are useful for encouraging of growth of sub-lethal bacteria and for the propagation of large numbers of bacteria i.e. for vaccine production. Liquid media are not usually used for primary isolation work, as it is difficult to obtain pure cultures and growth of contaminants in the sample may overtake that of pathogens. Solid media provide a platform on which individual colonies of bacteria can be identified and their characteristic properties studied. Bacterial species in mixed infections can be separated from each other. Semi-solid media are usually used for maintaining stock cultures, for propagating anaerobes and for motility testing.

Media can be broadly separated into categories according to their use. These include:

1. General purpose media that support a wide variety of organisms and are usually used when the identity of bacteria in a sample is unknown. Examples of general purpose media are nutrient broth, peptone water, trypticase soy broth, brain heart infusion broth, and blood agar.
2. Enrichment media are generally broths designed to promote growth of the micro-organisms present. They may or may not contain selective agents. An example is thioglycollate medium without indicator, which has a defined casein and soy peptone nutrient base.

3. Selective media contain inhibitors that permit the growth of certain micro-organisms while preventing the growth of others, e.g. selenite broth which is selective for Salmonella.

4. Transport media are usually low in nutritive value as they are designed to maintain the osmolality and moisture but provide little nutrition for the bacteria to grow.

5. Maintenance media serve to maintain bacteria; they therefore do not have nutrients whose products may be detrimental to bacteria such as acid produced from the metabolism of dextrose which will ultimately harm certain bacteria, e.g. Clostridium botulinum.

6. Differential or identification media. Bacteria demonstrate individuality in the nutrients they are able to use and the end products resulting from the metabolism of some of these nutrients can be used in the identification of bacteria. An example is MacConkey agar which enables the differentiation of lactose and non-lactose fermenters.

7. One-purpose media are highly selective and are designed to isolate a specific organism. Campylobacter medium contains a series of antimicrobials that allow the growth of C. jejuni but inhibits most other faecal flora.

SUPPLIES

- Sterile disposable plastic Petri dishes, 90mm and 65mm in diameter.
- Glassware including funnels, Schott bottles, conical flasks.
- Dehydrated culture medium. Due to the complex formulae and large range of media required, it is best to buy the media in concentrated form. Small laboratories might find it convenient to buy prepared plates. The variety stocked will depend on the requirements of the laboratory concerned. Table 1 contains a list of the most common media used.
- Citrated, oxalated or defibrinated equine, bovine or ovine blood.
- Analytical balance.
- Bunsen burner and butane gas (household gas).
- Tripod and porcelain gauze mat for Bunsen burner.
- Autoclave.
- Heat sensitive marking tape.
- Laminar flow cabinet.
- Magnetic stirrer.
- Water bath set to 45 - 50°C.
- Disposable filters, 0.22 and 0.45 µm.
- Plastic bags.
- Permanent marker pen.
- Refrigerator.
STORAGE OF DEHYDRATED MEDIA

Dehydrated media should be stored in a dry, dark place at room temperature (about 25°C). Its containers should be well sealed and tightly closed after use. Absorption of water leads to clumping and pH shifts.

Under ideal conditions, dehydrated media can be stored for at least five years after their manufacture. If a change in pH is observed after prolonged storage, it can be adjusted. Refer to the section on pH adjustment.

PREPARATION

Media prepared from dehydrated materials should be prepared in accordance with directions given by the manufacturer. The use of chemically clean glassware is essential. Aseptic techniques should be applied.

RECONSTITUTION

Dehydrated media should always be prepared with clean, freshly distilled or completely demineralized water that gives a neutral reaction. Distilled water that has been stored at room temperature for any length of time is apt to absorb gases sufficient to alter the final reaction and composition of the medium.

Rinse the containers (conical flasks or Schott bottles) with water to remove any traces of other substances. Chemical residues such as detergents can be responsible for bacterial inhibition and changes in reaction. The size of the vessel used should be such that the reconstituted culture medium can be thoroughly stirred, i.e. the volume of fluid medium should not fill more than three quarters of the container. Weigh out the desired quantity of dehydrated culture medium and add it to half the desired quantity of water. Mix thoroughly and then add the rest of the water, being careful to rinse down any material adhering to the walls of the vessel.

Culture media containing agar or gelatine must be heated in order to dissolve them completely. This can be carried out in a boiling water bath or over a Bunsen burner. The flask containing the medium should be frequently stirred during the heating process and should be removed from the heat once the agar or gelatine has dissolved. This is indicated by the fact that a viscid solution becomes clear and flows more easily with no particles sticking to the sides of the container. For large quantities of media, flowing steam or an autoclave may be used. Another method of preparation is to boil three quarters of the water. Suspend the dehydrated medium in the remaining cold water, taking care that the entire medium is thoroughly wetted and evenly distributed. The suspension is then added to the boiling water and the boiling continued until the medium has completely dissolved. This latter method decreases the preparation time. As all culture media are heat sensitive, they should not be heated longer than what is necessary.
PH ADJUSTMENT

If water at a pH of 7.0 and non-expired dehydrated commercial culture media are used, the pH should be within the specified limits. However, the pH of a batch of culture media should be monitored after sterilization. The pH is measured with either a pH meter or pH indicator strips. When using a pH meter make sure that compensation for temperature is made when standardizing the electrode. For media containing agar, pH measurement is done at 45 - 50°C (before solidification occurs) and liquid media at room temperature. The pH should always lie within the values advocated by the manufacturer of the medium.

How to correct the pH

a) Sterilize the reconstituted culture medium.

b) Remove a sample under sterile conditions.

c) Measure the pH of this sample and, if necessary, adjust to the correct value by titration.

d) Add the necessary volume of 1N or 1/10 N sodium hydroxide (NaOH) or concentrated hydrochloric acid (HCl) solution under sterile conditions.

STERILISATION

For most media of volumes of one litre or less, sterilization is carried out in an autoclave at 121°C for 20 minutes. Larger volumes may require 30 minutes or longer. Some components of a culture medium, such as urea, are heat sensitive. These have to be sterilized by filtration and added to the rest of the autoclaved culture media after cooling. Ensure that gelatine or agar based media are still in solution before autoclaving. The containers used should be able to withstand the heating process. Usually 1 liter Schott bottles are used, filled to no more than three quarters full to allow for expansion. These bottles are loosely capped or plugged with non-absorbent cotton wool during the heating process in order to allow the escape of gasses but not the entrance of steam. If conical containers are used they should be sealed with cotton wool plugs and overlaid with foil. In order to check whether the desired temperature was reached, heat sensitive masking tape should be placed on each bottle. After completion of the sterilization process, the bottles should be removed from the autoclave and rapidly cooled to pouring temperature, which, for solid media, is between 45 - 50°C. The vessels can be cooled under cold running water or in a water bath set at 45 - 50°C.

If an autoclave is not available, the media can be heated for 30 minutes at 100°C in a water bath or in a large pan filled with boiling water, on 2 or 3 consecutive days. Heat-resistant spores are thus induced to germinate and their vegetative forms subsequently destroyed with a relatively high degree of certainty.

Be careful not to over-sterilize as this will alter the composition of the medium, e.g. phenol red lactose broth under prolonged sterilization will produce large amounts of acid, and agar media will form a precipitate.

Proteins, such as blood, serum or egg are added to the sterilized medium after it has been cooled to 50°C to prevent their coagulation.
POURING MEDIUM INTO THE PLATES

The medium should be swirled or stirred with a magnetic stirrer before dispensing into Petri dishes, to ensure that it is evenly mixed. Pour the plates inside a laminar flow cabinet, if one is available. This will reduce the risk of contamination by bacteria and fungi to a minimum and allows for easy drying of the medium. The medium is usually poured into the Petri plates at between 45 - 55°C to obviate the formation of condensed water and to ensure that the fluid pours easily. Care should be taken while pouring to prevent the formation of froth or air bubbles. If air bubbles form they can be removed by briefly “fanning” them on the surface of the poured agar with the luminous flame of the Bunsen burner. After pouring a lid is placed on the Petri dish and the medium is allowed to solidify. At least 20 - 30 minutes are required.

If agar slants are to be made, the medium is poured into bottles or tubes which are then capped and placed lying down with the top of the bottle or tube elevated and allowed to solidify.

COLLECTION AND USE OF STERILE BLOOD

Equine, bovine or ovine blood is the most suitable for veterinary bacteriology. Blood from other species can also be selected, but if it is used, one must be aware that different haemolytic patterns, from those that form in media containing cattle or sheep blood, may develop. Horse blood is useful for the isolation of Haemophilus species as it is rich in X-factor, and for the demonstration of beta-haemolysis caused by the pyogenic streptococci. However, Rhodococcus equi and most strains of Staphylococcus intermedius are not beta-haemolytic when grown on media containing horse blood.

Sterile blood can sometimes be purchased commercially but if not, it can be obtained from a young animal which has not developed antibodies to the major veterinary pathogens or has been treated with antimicrobial agents.

1. Collection
   a) Practice a strict aseptic technique.
   b) Shave an area over a jugular vein.
   c) Wipe the area with a swab saturated in 70% ethyl alcohol and allow drying.

2. To prevent the blood from clotting one of the following methods may be used
   a) Collect into a pre-sterilized apparatus consisting of a tube leading into a conical flask containing glass beads (3mm in diameter). The flask is agitated continuously during collection and for at least 5 minutes after obtaining the blood. The defibrinated blood can be decanted into sterile bottles for storage in a refrigerator. The glass beads are recovered for reuse.
   b) Use of a blood donor kit and collection of blood into a bottle containing 0,2% sodium citrate.
PREPARATION OF CHOCOLATE AGAR MEDIUM

This is prepared by heating 10% blood agar at 80°C for 5-10 minutes. It is easiest to use a blood agar plate that is placed in an oven or incubator set at this temperature for 5-10 minutes. Heating of the blood causes rupture of erythrocytes and the liberation of their contents into the medium. It is used to culture X and V-factor requiring bacteria (Haemophilus) and other fastidious bacteria such as Campylobacter.

ACIDIC CULTURE MEDIA

Acidic culture media whose pH value is below 5.0 should be prepared under conditions of low temperature, as agar is hydrolyzed when heated in an acidic medium and gel stability of the culture medium thus declines.

POSSIBLE SOURCES OF ERROR DURING RECONSTITUTION AND HANDLING OF MEDIA

1. Clumping of dehydrated culture media
   a) Humidity too high during storage.
   b) Container was left open for too long.
   c) Container not tightly sealed after it had been opened.
   d) Dehydrated culture medium was too old.

2. pH shift
   a) Water was not neutral.
   b) Container not tightly sealed after it had been opened.
   c) Culture medium was overheated during reconstitution.
   d) Dehydrated culture medium was too old.

3. Turbidity, precipitation
   a) Water not adequately demineralised.
   b) Vessel used for reconstitution was not clean.
   c) pH value incorrect.
   d) Culture medium was overheated during reconstitution.
   e) In the case of self-mixed culture media, the basic components contained precipitating impurities.
   f) Caused by the sample material.

4. Temperature of solidification too high
   a) Too much dehydrated culture medium was weighed out and used.
b) Agar not stable.

5. **Gel stability too low**

a) Insufficient dehydrated culture medium was weighed out.
b) Dehydrated culture medium was not properly dissolved.
c) Culture medium was overheated, possibly at low pH value, during reconstitution.
d) Vessel was not swirled when pouring the plates.
e) In the case of self-mixed culture media, unsuitable or too little agar.
f) pH value was too low.

6. **Colour change**

a) In the case of culture media containing indicators, the pH was incorrect.
b) Vessel used for reconstitution was not clean.
c) Culture medium was overheated during reconstitution:
   - culture medium dark
   - coloured pigments destroyed
   - sugar caramelized

7. **Contamination**

a) Inadequate sterilisation.
b) Contaminated Petri dishes.
c) Contamination during pouring.
d) Air contamination.

8. **Growth too poor**

a) Residues of growth inhibiting substances present in vessels used for reconstitution (e.g. detergent), in the water, or in the sample material (e.g. antimicrobials).
b) Micro-organisms in the sample material already damaged.
c) pH shift.
d) In the case of culture medium bases, dose of additive incorrect.
e) Culture medium was overheated during reconstitution.
f) In the case of pour plates, temperature of medium was too high.
g) Incorrect agar depth.

9. **Growth too strong**

a) Culture medium overheated during reconstitution causing destruction of selective inhibitors.
b) In the case of culture medium bases, concentration of additives incorrect.
c) Culture medium was inoculated with too much sample material.


10. Colonies liquefy or spread

a) Surface of culture medium was too moist.
b) Surface of the culture medium was inoculated with too much sample material.
c) Culture medium was overheated during reconstitution causing destruction of inhibitors.

11. Atypical growth

a) Culture medium reconstituted incorrectly.
b) Dehydrated culture medium was too old.
c) Reconstituted culture medium was too old or unfit for use.
d) Wrong conditions were used for cultivation.
e) Residues of foreign substances present in the vessels used for reconstitution or for culture (e.g. detergents), in the water used, or in the sample material, (e.g. antimicrobials).

STORAGE OF RECONSTITUTED CULTURE MEDIA

The stability of prepared culture media is limited. If not otherwise specified, they can be stored for several months under appropriate conditions.

After solidification of the agar in the plates they should be sealed in plastic bags or individually taped and refrigerated at 2 - 8°C until use. Direct contact with light should be avoided. The plates should not be frozen as this destroys the gel structure. Plates can be stored at room temperature for 1 week.

In the case of media which contain unstable additives such as iodine, store the prepared medium base and mix in the additives just before use.

The culture media should be warmed to the required temperature by placing them in the incubator for several hours before use. If liquid media have been stored for a long time, it is recommended that before use they are heated to just below boiling temperature, to drive off any gasses and then rapidly cooled in a cold water bath or under a running tap. Do not agitate the media. The same method can be used for solid agar medium in a tube.

SAFE DISPOSAL OF CULTURES

The simplest way to dispose of cultures is to cover the entire surface of the culture medium with a disinfectant. The disinfectant should be allowed to act for at least 6 hours, or better still, overnight to ensure that all the micro-organisms are killed.

Bacterial cultures can also be destroyed by autoclaving them in plastic bags with a high melting point. If suitable incinerators are available, they can be burnt. When the micro-organisms have been killed and the culture medium has been inactivated, disposable plates can be thrown away. Glassware can then be cleaned and processed in the usual manner.
QUALITY CONTROL

The sterility and performance of all media must be verified before use, no matter whether they were prepared by an individual laboratory from “scratch” or from dehydrated materials or whether they have been received ready for use from commercial companies.

a) Date all materials on receipt and take note of expiry dates.
b) Several tubes or plates from each batch should be incubated to check for contamination.
c) For selective media, a 5 - 10 ml sample of each of these should be inoculated onto non-selective media and incubated at 37°C to check for contamination.
d) Each plate should be checked for surface contamination before use.
e) Media should also be examined for colour, clarity and evidence of dehydration. If abnormal they should be discarded. Certain media will form precipitates on standing. These should, however, disappear on heating.
f) The pH of each batch should be checked after sterilization (See earlier section).
g) Each batch should be subjected to performance testing using stock cultures with typical cultural characteristics. Performance testing is based upon the use for which the medium was intended and should include bacteria that give both positive and negative results.

REFERENCES


5. The Oxoid Manual
Table 1: Commonly used culture media for microbes of veterinary importance

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Culture media</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-selective for most non-fastidious bacteria</td>
<td>Nutrient broth or agar</td>
<td>Used as a basal medium to which inhibitory or growth promoting substances can be added. Used for testing biochemical reactions</td>
</tr>
<tr>
<td></td>
<td>Peptone water</td>
<td>Used as a basal fluid for the growth of pure strains of bacteria, for testing biochemical reactions by adding various sugars and for indole production</td>
</tr>
<tr>
<td></td>
<td>1% Glucose or 10% serum broth</td>
<td>Promotes growth of fastidious bacteria</td>
</tr>
<tr>
<td></td>
<td>Blood agar base to which 5-10% citrated blood is added</td>
<td>Promotes growth of fastidious bacteria. Differentiation according to type of haemolysis</td>
</tr>
<tr>
<td></td>
<td>Columbia agar base</td>
<td>High quality complete media for the cultivation of fastidious organisms; can be used as bases for preparing various special culture media</td>
</tr>
<tr>
<td></td>
<td>Brain heart agar or broth</td>
<td>For fastidious bacteria and fungi. Usually in slants</td>
</tr>
<tr>
<td></td>
<td>Tryptose agar</td>
<td></td>
</tr>
<tr>
<td>Anaerobes</td>
<td>Fluid thioglycollate medium</td>
<td>Used for the growth and fermentation of anaerobes, especially for those micro-organisms which form spores. Can also be used for Campylobacter isolation</td>
</tr>
<tr>
<td></td>
<td>Cooked meat broth</td>
<td>Especially for Gram-negative anaerobic micro-organisms which do not form spores.</td>
</tr>
<tr>
<td>Brucella</td>
<td>Tryptose broth</td>
<td>For enrichment</td>
</tr>
<tr>
<td></td>
<td>Brucella agar</td>
<td>For cultivation</td>
</tr>
<tr>
<td></td>
<td>Tryptose Agar</td>
<td>For cultivation</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Rappaport broth and agar</td>
<td>Selective for salmonellas</td>
</tr>
<tr>
<td></td>
<td>Selenite enrichment broth</td>
<td>Selective for salmonellas</td>
</tr>
<tr>
<td></td>
<td>XLD/XLT agar</td>
<td>Weakly selective. <em>Salmonella</em>, <em>Proteus</em> and <em>Citrobacter</em> spp. form black colonies. XLT: <em>Salmonella</em></td>
</tr>
<tr>
<td></td>
<td>Triple sugar iron agar</td>
<td>For identification</td>
</tr>
<tr>
<td></td>
<td>MacConkey agar without cristal violet</td>
<td>Weakly selective and detects lactose fermenters</td>
</tr>
<tr>
<td></td>
<td>Peptone water</td>
<td>For preliminary, non-selective enrichment, particularly of sublethally damaged <em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>Dermatophytes</td>
<td>Sabouraud 2% dextrose agar</td>
<td>Non-selective</td>
</tr>
<tr>
<td></td>
<td>Dermatophyte selective agar</td>
<td>Inhibitory to most bacteria and saprophytic fungi. Contains an indicator to demonstrate early growth</td>
</tr>
<tr>
<td>Fungi</td>
<td>Malt extract agar</td>
<td>For isolation and enumeration, particularly of yeasts and other fungi</td>
</tr>
<tr>
<td></td>
<td>Sabouraud dextrose agar</td>
<td>For the cultivation, isolation and identification of fungi</td>
</tr>
<tr>
<td>Culture Medium</td>
<td>Purpose</td>
<td></td>
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<tr>
<td>-------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Potato dextrose agar</td>
<td>For cultivating, isolating and counting yeasts and other fungi.</td>
<td></td>
</tr>
<tr>
<td><strong>Enterococci</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin-Esculin bile agar</td>
<td>Selective for enterococci</td>
<td></td>
</tr>
<tr>
<td>6.5% Salt agar</td>
<td>To distinguish non-enteric Group D streptococci.</td>
<td></td>
</tr>
<tr>
<td><strong>Haemophilus/Histophilus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Columbia agar</td>
<td>Non selective, nutrient-rich agar</td>
<td></td>
</tr>
<tr>
<td>Chocolate agar</td>
<td>Used to culture fastidious bacteria such as Haemophilus/Histophilus and Campylobacter. Will also aid in the production of a capsule by Cryptococcus neoformans</td>
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</tr>
<tr>
<td>Haemophilus selective agar</td>
<td>Selective</td>
<td></td>
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<tr>
<td><strong>Lactobacilli and Megabacteria</strong></td>
<td></td>
<td></td>
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<tr>
<td>MRS agar</td>
<td>For all Lactobacillus species</td>
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<tr>
<td>Tomato juice agar base</td>
<td>Selection of lactobacilli</td>
<td></td>
</tr>
<tr>
<td><strong>Listeria</strong></td>
<td></td>
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<tr>
<td>Tryptose agar and broth</td>
<td>Non-selective, nutrient-rich.</td>
<td></td>
</tr>
<tr>
<td>Listeria enrichment broth base</td>
<td>For the enrichment of Listeria monocytogenes</td>
<td></td>
</tr>
<tr>
<td>Listeria selective agar</td>
<td>For the selective isolation of L. monocytogenes</td>
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<tr>
<td><strong>Mycobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowenstein-Jensen medium base</td>
<td>Selective for M. tuberculosis complex</td>
<td></td>
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<tr>
<td><strong>Mycoplasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayflicks broth</td>
<td>For the enrichment of mycoplasmas</td>
<td></td>
</tr>
<tr>
<td>Hayflicks agar</td>
<td>For the growth of most mycoplasmas</td>
<td></td>
</tr>
<tr>
<td>Chalquest agar</td>
<td>For the growth of mycoplasmas that will not grow on Hayflicks medium.</td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococci</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mannitol salt, phenol-red agar</td>
<td>Selection and differentiation of mannitol positive staphylococci</td>
<td></td>
</tr>
<tr>
<td>DNase test agar</td>
<td>Detection of microbial DNase</td>
<td></td>
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<tr>
<td>Baird-Parker medium</td>
<td>Isolation and recognition of coagulase positive staphylococci</td>
<td></td>
</tr>
<tr>
<td><strong>Streptococci</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edward's modified blood agar</td>
<td>Isolation and identification of streptococci isolated from bovine milk</td>
<td></td>
</tr>
<tr>
<td>Phenylethyl alcohol agar (BBL)</td>
<td>Useful for isolation of material contaminated with Proteus spp. and other Gram-negative bacteria</td>
<td></td>
</tr>
<tr>
<td>TKT/FC Medium (Gibco)</td>
<td>On this medium S. agalactiae produces light coloured colonies with a relatively large β-haemolytic zone.</td>
<td></td>
</tr>
</tbody>
</table>
### Antimicrobial susceptibility tests

<table>
<thead>
<tr>
<th>Culture Media</th>
<th>Method</th>
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<tr>
<td>Mueller Hinton agar</td>
<td>Plate diffusion method</td>
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<tr>
<td>Mueller Hinton broth</td>
<td>Serial dilution method for aerobic bacteria</td>
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<tr>
<td>Isotonic sensi-agar</td>
<td>Plate diffusion method</td>
</tr>
<tr>
<td>Anaerobic TVLS medium base</td>
<td>Serial dilution technique for anaerobes, including those requiring complex nutrients</td>
</tr>
<tr>
<td>Sabouraud 2% dextose broth</td>
<td>Assay for antifungal agents</td>
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</tbody>
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### Media used for the identification of bacteria

<table>
<thead>
<tr>
<th>Culture Media</th>
<th>Use</th>
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<tbody>
<tr>
<td>Christensen citrate agar</td>
<td>For detecting citrate degradation; particularly for differentiating between <em>Shigella</em> and lactose negative <em>Escherichia</em> variants</td>
</tr>
<tr>
<td>Simmons citrate agar</td>
<td>For the detection of citrate degradation</td>
</tr>
<tr>
<td>Triple iron sugar agar</td>
<td>For the identification of the members of the family <em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>Christensen urea agar base</td>
<td>For the detection of micro-organisms which metabolise urea, particularly weak urease producers</td>
</tr>
<tr>
<td>Lysine iron agar</td>
<td>For the simultaneous detection of lysine decarboxylation and hydrogen sulphide production</td>
</tr>
<tr>
<td>MR-VP broth</td>
<td>For the methyl-red and Voges-Proskauer tests</td>
</tr>
<tr>
<td>Nutrient gelatin</td>
<td>For detecting organisms which degrade gelatin</td>
</tr>
<tr>
<td>Nitrate broth</td>
<td>For detecting nitrate reduction and indole production, the relevant reagents must be added</td>
</tr>
<tr>
<td>OF Basal medium base</td>
<td>For the detection of the oxidative and fermentative degradation of carbohydrates</td>
</tr>
<tr>
<td>Phenol-red broth base</td>
<td>Culture medium base for biochemical differentiation.</td>
</tr>
<tr>
<td>Rice extract agar</td>
<td>For the differentiation of yeasts on the basis of the formation of chlamydospores and pseudomyccelia</td>
</tr>
<tr>
<td>SIM Medium</td>
<td>For the simultaneous detection of sulphide and indole production, and motility</td>
</tr>
<tr>
<td>Tryptone Water</td>
<td>For the detection of indole production.</td>
</tr>
</tbody>
</table>