Anaerobic Agar

For the cultivation of anaerobes, specially of Clostridium species

Formula in grams per liter:

Casein peptone	17,50	Soy Peptone	2,50
Sodium Chloride	2,50	L-Cystine	0,40
Destrose	10,00	Sodium Thioglycollate	2,00
Sodium Sulfoxyl Formaldehyde	1,00	Methylene Blue	0,002
Bacteriological Agar	15,00		

Final pH: 7,2 ± 0,2 at 25 °C

Preparation:

Suspend 51 grams of the medium in one litre of distilled water. Soak for 10-15 minutes. Mix well and heat with agitation. Boil for one minute or until the medium is completely dissolved. Sterilize in the autoclave at 121°C (15 lbs. sp.) for 15 minutes. The medium can be incubate in anaerobes jar or with Brewer lids for anaerobiosis.

Uses:

Three reducing agents generate an strong and stable descent of the oxidation-reduction potential, thus securing good anaerobic conditions. Methylene blue acts as the redox indicator.

The seeding of the sample (clinical or food) can be performed by surface inoculation or by emptying. That is, by inoculating and mixing the product to study with the medium, melted and cooled to 45-50°C. Normally the sample should never be heated to destroy the vegetative forms of the anaerobe, as the anaerobes non sporeformers will be also destroyed. Nevertheless, sometimes it would be useful to heat the sample when sporeformers such as Clostridium are sought, except C. Perfringens, which rarely forms spores. When heating is indicated, warm the sample suspended in a liquid diluent (peptone water, buffering phosphate solution, etc.) for 10 minutes between 70°C-80°C.

The plates of Anaerobic Agar can also be incubated in a normal atmosphere covering the surface of the plates with a Brewer lid. In this case, it is important to leave about 1,5 cm on the outer edge of the plate uninoculated. With care place the Brewer lid on the plate to obtain a hermetic seal. The central part of the lid should not touch the surface of the plate but form a chamber of 2-5 mm.

When growth is observed, open the plate and pick the desired colonies. Incubate longer if necessary. If the medium has not been prepared shortly above the surface. before its use, it is necessary to heat and remelt it to expel the dissolved oxygen.

If for some reason the sample can not be streaked on the Anaerobic Agar plate, place the sample in Thioglycollate Medium without Indicator previously heated and cooled. Incubate until the next day and seed the Anaerobic Agar plate. Thioglycollate Medium without Indicator is an excellent enrichment broth and frequently this method gives better results than direct seeding.

Microbiological Tests:

Microorganisms	Growth
Clostridium butyricum ATCC 9690	Good
Clostridium perfringens ATCC 12919	Good
Clostridium sporogenes ATCC 11437	Good