AGAR PLATE TEACHER'S NOTES

The Petri Dish

The Petri dish is one of the most important tools in microbiology. It was invented by Julius Petri. The loose-fitting lid protects the agar from unwanted contamination. It is important to grow microorganisms (bacteria and fungi) on solid media so that we can look at the colonies. We also use liquid media which go cloudy when there has been lots of growth, but we can't tell which microorganisms are growing. Agar plates let us look at the individual colonies so we can check what is in our culture.

Before agar was used, microbiologists were using gelatin to solidify culture media. The problem with gelatin is that for many microorganisms it is a food source. This meant that in the late 1800s microbes were liquefying the solidifying agent! A pioneer in microbiology named Fannie Hesse suggested that agar could be used instead of gelatin. This change immediately solved the problem because there are very few organisms that can degrade agar (which originally comes from seaweed). Agar remains the most popular solidification medium for microbiology to this day.





THINGS TO CONSIDER

Heating and cooling

When making agar, we heat it to around 100°C. This makes it easier to dissolve the agar and the bouillon, but it also sterilises all the ingredients that will be added. If we didn't do this, then microorganisms naturally present in the bouillon, the agar and the containers would start to grow immediately. Therefore, we need to heat everything up to kill all the microbes and then keep the containers sealed so that no contaminating microbes can get on to our Petri dishes.

We then cool the agar to 50°C before pouring, this is to make it easier to pour and the agar will not be able to solidify at 50°C.

Cleanliness

The Petri dishes are an ideal place to grow microorganisms, and microorganisms are all around us. If you leave one of the dishes open for too long then microbes from the air or your breath will land on the Petri dish and contaminate your experiment. Therefore it is important to make sure that they are kept closed most of the time, and only open them when you are ready to add your microbial sample.

Once microbes have grown on the Petri dishes, it is also important to protect yourself from the microorganisms that grow there. From the time you take your sample, to the time that you see microbes growing on the surface, there could be over 1 million more microbial cells present. Be sure not to leave the lid off your Petri dishes and don't sniff or touch the microbes that have grown.

Beef bouillon

Beef bouillon or variations of this are still a very popular growth medium for microorganisms to this day. However microbes grow in different ways when exposed to different food sources. For example, chicken, fish or vegetable bouillon may give different results.

Beef bouillon is an example of a "complex medium". It is very rich in nutrients and many different types of microorganisms will grow there. This means that fast growing organisms will dominate and use up nutrients faster so that slower growing organisms don't get a chance to grow. In microbiology labs, researchers also use "selective media" which can only be used to grow a very small number of different microorganisms. For example, adding salt to growth media can encourage the growth of bacteria that reside on the skin and discourage organisms from the environment. Often, researchers will grow the same sample on both complex and selective media to try and fully understand the microorganisms that were present.

It is important to remember that the growth media that we use often doesn't closely resemble the original environment in which the microorganisms were found. For example, if you sample the plug in your sink, it does not closely resemble beef bouillon. For this reason, we need to be careful about inferring too much about what we see in the Petri dish to what is happening in real life. The organism that looks very common on the Petri dish, may not be as common in the plug hole.



Spills

All spills should be reported and dealt with by an adult. Liquid disinfectant (eg. sodium chlorate(I) (hypochlorite) of greater than 1% mixed with water) should be applied to the spill and cleaned with disposable paper. The adult cleaning the spill should wear disposable plastic gloves. If any of the spill gets on your hands, wash them thoroughly with soap and water.

Disposal

Anything that has come into contact with microorganisms must be disposed of carefully and in accordance with your local guidelines. Please check these before starting any cultures.

In general, there are two acceptable methods for killing microorganisms prior to disposal.

Autoclaving

The autoclave is a specialised sterilisation device applying high temperatures and pressures that most organisms cannot withstand. If your school department has access to an autoclave, use this to sterilise the finished petri dishes before disposal. Be sure to use autoclavable discard bags and follow the manufacturer instructions.

Chemical sterilisation

A chemical disinfectant that does not lose activity in the presence of organic matter can be used to sterilise the agar plates before disposal. There are many different alternatives and brand names but please check that they are suitable for disinfection. An example is a solution of sodium chlorate(I) (hypochlorite) of greater than 1% mixed with water. Leaving organic matter here for 15 minutes will sterilise it.





