My name is Doctor Sapna Gaitonde and I'm assistant

professor at Government, College Of Arts, Science and Commerce,

Condola. Today we are going to do unit 3 that

is bacteriological techniques and the module name is

pure culture isolation.

So what are you going to study in this particular model is.

First of all, how to isolate the pure bacterial culture.

Secondly, the methods and skills that are required in

isolation of the organisms from a mixed culture.

At the end of this module you will be able to explain the

aseptic techniques in isolation of pure cultures and

also you will be able to explain the different types of

streaking methods.

So let us start with pure culture isolation. Now most of

the environment they carry mixed microbial population. So it is

important for us to 1st dissect this mixed microbial population

and see that we are isolating the bacteria from this

population. Now there are different objectives as to why

pure culture isolation is important. First of all, it is

very important to demonstrate a good aseptic technique. You will

also learn the skill as to how to isolate the pure culture and

Thirdly to isolate the microorganisms from wide variety of sources. So these are a few objectives of pure culture isolation. Now a single colony that has been isolated is transferred into the fresh medium to obtain a larger homogeneous culture, which can later be studied and characterized using a variety of methods.

Now the old aseptic technique that we're going to use for the isolation of the culture involves certain conditions that need to be fulfilled. As you can see on the slide, the conditions are the first one is the area of the table on which you're going to do this aseptic technique of isolation of your culture has to be wiped with an antiseptic and kept very much clean and sterilized. Secondly, the instruments that go into this aseptic technique needs to be sterilized, which will also involve sterilizing the inoculating nichrome loop. The media in which the culture has been inoculated before inoculating the culture, the culture. Also, the medium has to be sterilized, and later the culture has to be inoculated.

All the culture materials which are there, they all need to be covered with proper leads and caps. This is all to minimize

the potential contamination that can take place.

Now the media that you can use for isolation of the cultures could be nutrient Agar. It could be Luria Burtani agar or trypticase soy Agar.

Now let us see what are the different methods for obtaining pure cultures. So there are five more important methods. The the first one is the streak plate method. The second pour plate method. The third spread plate method, the 4th serial dilution method, and the 5th enrichment culture method. Now let us see each one of these method or little in detail.

This is the first method that is a streak plate method. Now in this method is the most commonly used method. You you take the solid Agar medium, say Nutrient agar. You have taken and you need a nichrome loop to inoculate them your culture so you have to 1st sterilize the nichrome loop. You have to take the culture after cooling the loop. We have to take a loop full of the inoculum and you to start streaking the culture on the Agar plate.

As it is shown in the diagram.

Our annual streaking. It also have to make sure that in

between your sterilizing after every three lines of streak,

you're sterilizing the loop and you're not re inoculating it.

You're taking the culture from that streak where you have

finished and you're pulling it on the other side.

So consequently what will happen is the inoculum in these streets

will thin out and because of which you will finally get those

pure qualities after incubation of the medium.

Now, this method involves two different types of streaks. One

is the quadrant streak, second one is that T- streak.

So let us see the second method and the third method that is

spread plate and the pour plate method. Now as you can see in

the diagram. The pour plate on the top where in what you're

doing is you're taking some amount of inoculum and you are

mixing it with the melted Agar Medium. The melted Agar medium

which is there has to be sterile and also make sure that the

temperature of this is cool down from a very high temperature of

autoclaving to around 42 to 45 degrees and then you put your

culture in that mix well and then pour in the sterile.

plate under aseptic conditions. Then you can

incubate the plates.

So you have to make sure that whatever inoculum that you're

using, you're directly putting it in the medium and this

medium needs to be in between 42 to 45 degrees only. It

cannot be hotter than that and be nobody to degrees the other

starts so define.

Next to the spread plate technique. As you can see in the

diagram. You have to take the solid Agar medium. Then you have

to take some amount of inoculum,say around .1 mL using a

sterile pipette, and place it in the center of the medium and

then you have to use a stirrer or you can use a bent glass

rod that you can see in the picture. Also, before using

that, you have to sterilize it using say alcohol to flame it

cool down and then spread this inoculum of 1 ML.

Approximately on the entire surface of the Agar.

Later on you can dry it a little bit and incubate.

So after incubation of the Agar Plates you will find that some

colonies have been isolated, so this is how we have to isolate

the pure culture.

Next method is a serial dilution method. As you can see in the

diagram, there are a variety of tubes your and there is one

particular tube which you can label is 10 to the power zero,

which is your original inoculum from this you have to transfer

some amount of the inoculumOK into the other tubes. So

suppose that you have taken the sterile medium say water and if

you have taken say your inoculum, whatever the soil,

culture or anything.

And you have to make serial dilutions in this water. Or you

can take it in the broth.

So how you will make this dilution, say 9 ML of broth.

You have used an. You're using the inoculum of 1 ML.
So 1 ML has to be put in this9 ML and shaken. Well then from

this tube which you have inoculated just now, which had

9 ML of the broth, you have to label this 10 to the

power minus one. Then you have to take another broad tube.

Which you have to label as 10 to the power minus two and then make a

transfer again as 1 ML from10 to the power minus one to 10

to the power minus two and so on. So at the end of the

dilutions you will have the very well isolated colonies that

you'll be able to see.

And the last method is the enrichment culture method. Now

this method basically is derived for the bacteria which

needs some special nutrient in Agar medium. Or they need

some special physical condition for their growth. Some of the

examples are given here that is media with high salt

concentration to select thehello files. Second one selenite

broth which is used to select Salmonella species and the third one is alkaline peptone water

for cultivation of Hebrew.

These are some of the references from which the content has been

taken. Thank you very much.