I am Ruella D'souza today under Unit 1, the normal microflora of

the human body and host pathogen

interaction. We will be studying Module 11 titled Toxigenicity.

The outline of the module.

We will be studying what is an endotoxin?

How is it detected in a laboratory?

What are the components of LAL assay kit?

How is the Lal Assay carried out?

At the conclusion of this

presentation. The student will be able to.

Comprehend the significance of

the LAL assay. Identify its different components and their

role. Understand the principle of the LAL assay.

We need to have a quick recap of what you have studied in

the earlier module.

The role played by Endotoxin and Exotoxin in host

pathogen interaction has already been covered.

The endotoxin Toxigenicity is due to a heat stable LPS

that is a lipopolysaccharide.

Now lipopolysaccharides are found in the cell wall of Gram

negative bacteria. And this endotoxic activity is

dependent on the lipid A component of LPS.

The key points we need to remember about the endotoxin.

It is not secreted outside the

bacterial cell. Because they are part of the cell wall itself.

Endotoxins cannot be toxoided.

What does this mean? A toxoid is an immunogenic version of a

toxin, but it is inactive.

An endotoxin cannot be made a toxoid.

Endotoxins are rather poor antigens, an active only in

large doses.

Large quantity of endotoxin can cause

dangerous changes in the human body, which can lead

to systemic shock which can prove fatal.

Endotoxins have been the bane of the medical industry. The

presence of an endotoxin on various medical devices that are

inserted in the body and in the Pharmaceutical industry can be

potentially life threatening.

There is thus the need for sensitive, accurate in vitro

tests in order to detect their presence.

The lab assay is the answer to all of this. The Limulus

Amebocyte lysate assay.

The Limulus Amebocyte Lysate assay. Gets its name from the horseshoe crab Limulus Polyphemus. It has a clot protein in its amoebocytes which is obtained and used in the LAL assay. You have a picture of theAtlantic horseshoe crab to the right of your screen.

Now let us go to the assay and how it works.

The assay contains a pro clotting enzyme.

This pro clotting enzyme is the inactive version of an enzyme.

The kit also contains procoagulogen and calcium.

Now, endotoxin from a sample.

Reacts in the presence of calcium with the pro clotting enzyme to form the active version, which is called the clotting enzyme. This active clotting enzyme is then responsible for converting procoagulogen into coagulogen.

Coagulogen has various sub units. These subunits then join via disulfide bonds to form a gel clot.

So let us go through the principle once more.

To oversimplify it, the endotoxin combines with the clot protein and then forms a gel clot, that is the basics.

The kit as we have just covered contains calcium pro clotting enzyme and pro coagulogen.

How do these work?

The pro clotting enzyme is activated by the bacterial endotoxin. These may be present in a device that is going to be used in future or in pharmaceutical drugs. The presence of calcium will enable the formation of an active clotting enzyme. The active clotting enzyme will catalyze the cleavage of pro coagulogen into the polypeptide subunits which we term coagulogen.

And the joining of these subunits will form a gel clot.

Now this gel clot can be quantitated spectrophotometrically.

The removal of endotoxins presents more of a problem than their detection, so in case endotoxins are detected on glassware or medical devices,they can be inactivated. If said device is heated at 250 degreesCelsius for 30 minutes.

Currently companies are developing special filtration systems and filtration cartridges that would retain endotoxins and thus ensure the devices are safe for use.

Now that we have studied all that we know about LAl assay, we come to a point of revision.

We have in front of us a

reflection spot. There are somethings on the screen that aren't

marked. Could you answer the questions I have for you?

What does the red ribbon signify?

Can you think about that?

If you thought it was endotoxin,then you're absolutely right.

Question number 2.

Can you state the inactive form of the enzyme?

If you answered pro clotting enzyme, then you are correct.

Finally, question #3.

Can you identify the block marked with a question mark?

The answer is coagulogen.

You can refer to any of the prescribed textbooks, including

AnanthNarayan and Paniker's Textbook of microbiology.

Javitz Melnick and Adelberg

medical microbiology. Mims Medical microbiology and

immunology. And Prescott and Kleins microbiology.

Thank you.