Enzyme Science and Technology
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Module - XI
Enzyme Applications (Part-I)
Lecture - 45
Application of Enzyme (Part-II: Medical Field)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme. In the course Enzyme Science and Technology and in this particular module, we are discussing about the Application of Enzyme in the different fields.

And what we have discussed? We have discussed about the application of the enzyme in the food industry. So, if you recall in your previous lecture, we have discussed about the role of proteases, pectinases, alpha amylases and so on in the different types of the you know food industries right. So, food apart from food industry, the enzymes are also having the diversified applications into the medical field right.

And in the case of medical field, the enzymes are being used for the diagnostic purposes and as well as enzyme being used as a target to develop the different types of medicines. So, in the current lecture, we are going to discuss about how the enzyme can be a diagnostic marker into the diagnostic marker and how you can be able to do the enzyme assays for the different enzymes to detect and predict the functional role or the pathophysiological status of a particular organ.

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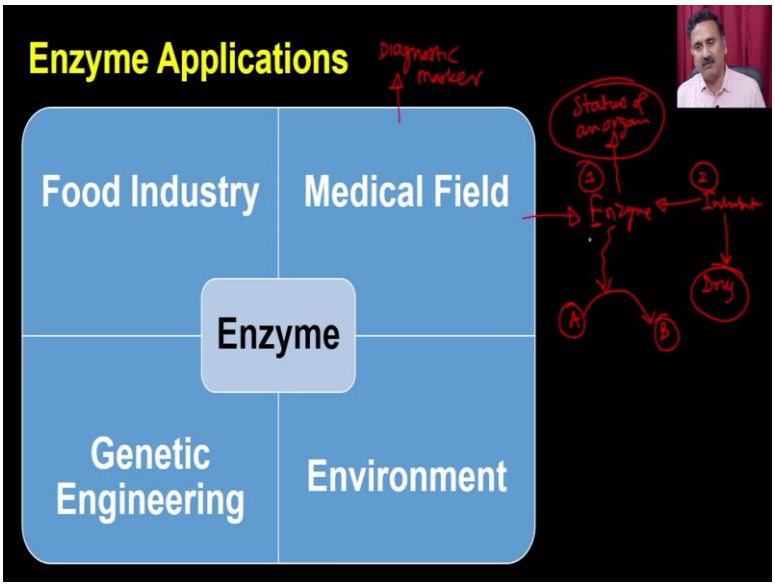

So, as we say that in this particular module, we are going to discuss about the application of the enzyme into the food industry, medical field, genetic engineering and environment. So, let us start discussing about the application of the enzyme into the medical field. So, within the medical field, the enzyme can have the different roles. For example, they can be a diagnostic marker.

They could be the enzyme for which you are actually going to target and develop the inhibitor and these inhibitors will nothing but going to be called as drug or in some cases. The enzyme itself is being required catalyzed the conversion of A to B and in that case the enzyme itself is going to be a therapeutic molecule.

So, these are the 3 important aspects, one where the enzyme is going to be a therapeutic molecule, second the enzyme is going to be a utilized by the inhibitor and that is why it is actually going to be resulted into the production of drug and the third aspect is that the enzyme itself is actually going to give you the status of a particular status of an organism on organ right. Because, the enzymes are present in the organs, so they can actually be able to give you the status of that particular organ functioning.

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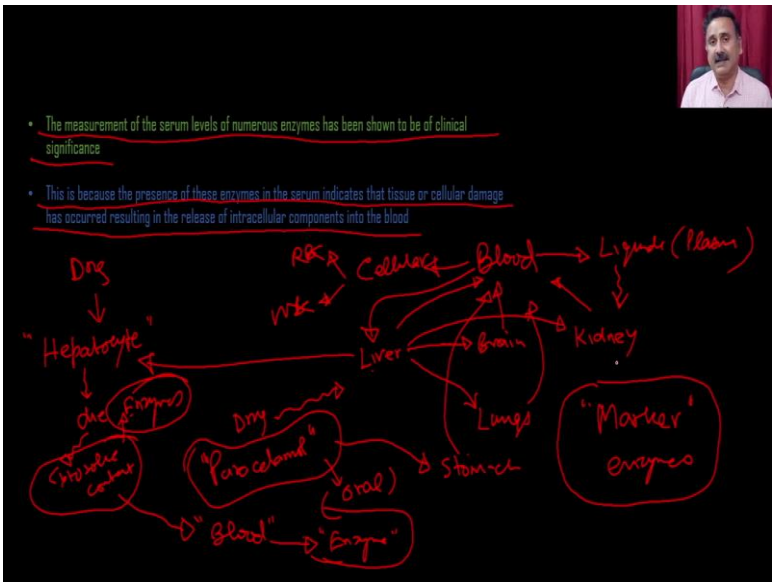

How it happens? The measurement of the serum level of various numerous enzymes that has been shown to be a clinical significance this is because the presence of these enzymes in the serum indicate that the tissue or the cellular damage that occurs resulting into the release of intracellular component into the blood. So, this is what exactly happens, right. So, all the organs are actually communicating with each other with the help of the blood, right and the blood is actually having the 2 component.

One, you have the cellular component, right; where you have the different types of cells like RBC, you can have the WBCs and so on. The other component is the liquid component, right or which is also being called as the plasma, right. And the plasma is actually the components which communicate between the different types of organs.

So, you can imagine that in a human body, what you have? You have the different types of organs, you can have the liver, you can have the brain, you can have the kidney right, and you can have the lungs and so on right.

All these organs are you know are communicating with each other with the help of the blood, right. Because they are sending all their component into the blood and that is how they are actually. So, imagine the situation that you are taking a drug, right. For example, you are taking a drug, for example Paracetamol ok. I am just giving you an example, it could be just for the experiment a example purpose here ok.

So, for example, you have taken the Paracetamol ok. So, how you are going to do? You are going to take the Paracetamol orally right. So, it what will happen? Paracetamol will go into the stomach right and from the stomach it will absorb and then from the stomach it will go into the blood, right.

From the blood, it will first go into the liver and from the liver then it will actually again enter into the blood and through, they are actually going to go into the different organs, right. It will go into the lungs, it go to brain, it goes to the kidney, it goes to heart and other places.

But imagine a situation that you have taken a drug which goes into the stomach and from the stomach, it goes into the blood and from the blood, it goes into the liver. Now, this drug is actually little cytotoxic, right. So, what happen is that it is actually going to start causing the problem to the liver cells. So, if you see the liver, the liver is made up of a cell which is called as hepatocyte, right. What will happen is it is starting killing the hepatocytes.

So, if it is hitting the, it is causing the toxicity to the hepatocytes, the hepatocytes will die ok and as a result they will actually going to release their cytosolic content, right. Which means and once they release the cytosolic content, that cytosolic content is actually going to get release into the blood; which means, cytosolic content means the different types of enzyme right and that means all the enzymes will enter into the blood.

So, if imagine that you are actually taking a drug and that drug is causing the death of the hepatocytes, then that hepatocyte content is actually going to be present into the blood. So, now imagine that if I have an enzyme which is specific for the hepatocytes, I can detect, if I detect that particular enzyme into the blood. So, for example, if I detect that particular enzyme, I can very crucially or I can very confidently say that there is a problem to the liver, right?

Same is true for the other worms also. Same is true for the kidneys, same is true for the brains, same is true for the lungs, same is true for the heart. So, this is the basic idea of how you can actually be able to detect the serum level of the enzyme and that actually is indirectly going to tell you that there is a problem to that particular organ.

Because all the organs have their specific enzyme and all these enzymes are called as the marker enzymes ok. Marker enzyme means they will not be present or they will not be present in high quantity in the other cells.

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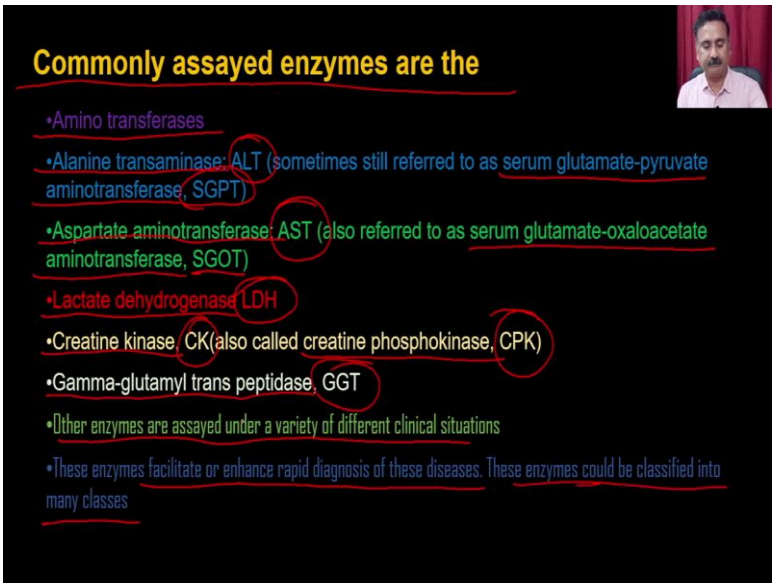

So, what are the different enzymes? What you going to actually be able to use for this particular purpose? You can actually be used the amino transferases, you can have the alanine transferases or ALT. If the ALT is present into the serum, then it is also called as the SGPT, right. Then it is called as serum glutamate pyruvate amino transferases. Then we also have the aspartate amino transferases, which is called as AST. It is also known as the serum glutamate oxaloacetate amino transferase or SGOT.

So, and then we also have the lactate dehydrogenase, short form LDH. Then we have the creatine kinase or CK. It is also known as the creatine phosphokinase or CPK. Then we also have the gamma-glutamyl transpeptidase GGT and you also have the other enzymes that are also been assayed under the different clinical situations. These enzymes facilitate the enhance rapid diagnosis of these diseases. The enzyme could be classified into the many classes.

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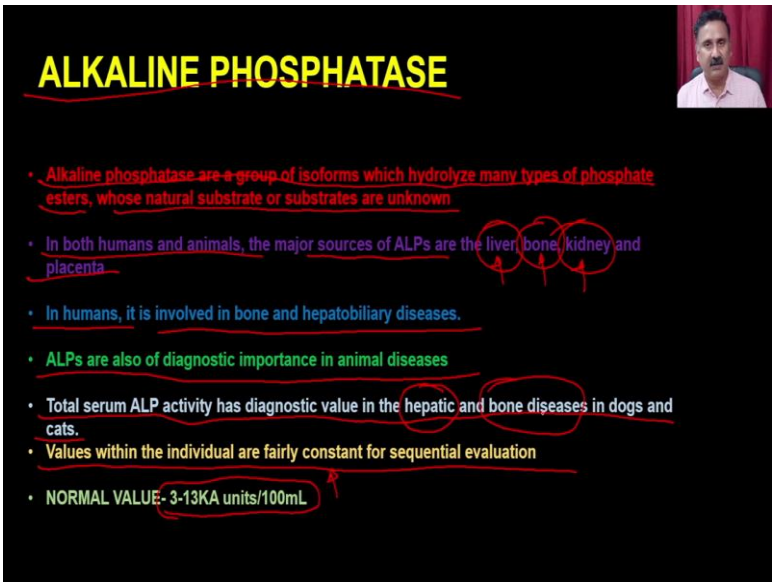

So, the first enzyme what we are going to discuss about the Alkaline Phosphatase. So, alkaline phosphatase are the group of isoforms which hydrolyze many types of phosphate esters whose natural substrate or the substrate are unknown. In both human and animal, the major source of alkaline phosphatase are the liver, bone, kidney and the placenta.

Which means these are actually the organs which are if you see the serum level of alkaline phosphatase very high, then you will say that there could be a problem into the liver, bone, kidney or to the placenta ok. In human, it is involved in the bone and hepatobiliary diseases. Alkaline phosphatases are also of diagnostic importance in the animal diseases. For example, the total serum ALP activity can be a diagnostic value in the hepatic and bone diseases in the bone in the case of dog and cats.

So, it actually predicts the any kind of abnormality or pathological situation of the liver as well as the bone diseases. Values within the individuals are fairly constant for the sequential evaluations. The normal level of the alkaline phosphatase activity is between the range of 3 to 13 units per 100 ml ok. So, if the values are above to this, then you will say that there will be a problem into the liver and as well as the bone.

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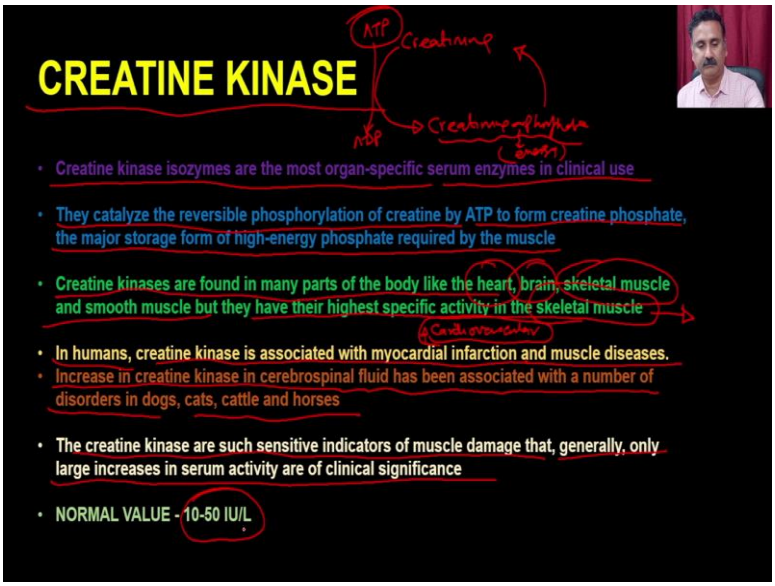

Then the second is Creatine Kinase, the creatine kinase isoenzymes are the most organ specific serum enzyme in the clinical use. They catalyze the reversible phosphorylation of creatine by the ATP to form the creatine phosphate. The major storage form of the higher energy phosphate required by the muscles so, what reaction creatine kinase is catalyzing is that it is converting a creatine into creatine phosphate and it is actually going to utilize the ATP.

So, it is actually going to take up the ATP and reduce the ADP and in this process, it is actually going to produce the creatine phosphate and that is how it is actually going to produce the high energy derivatives. This creatine phosphate is again going to be get converted into creatine and that is how this high energy phosphate group is going to be broken and that is how it is actually going to release energy.

And that energy is actually going to be utilized for facilitating some of the reactions. So, creatine phosphate is actually a high energy you know phosphate group or phosphate compound which can be able to use to carry the energy from one place to another place just like ATP, right. ATP is also like that.

So, creatine kinase are found in many parts of body like the heart, brain, skeletal muscles and the smooth muscles, but they have their highly specific activity in the skeletal muscles. Which means if there is a problem in the skeletal muscles, the creatine kinase

level in the serum is actually going to go off. So, in humans, creatine kinase is associated by the with the, is associated by with the myocardial infraction and the muscle disease.

Myocardial infraction is a cardio vascular disease. So, it is a disease where the heart muscles are actually going to be get affected. So, it is actually going to in general, the creatine kinase is actually going to give you the indication that there is a problem with the muscular disorders or muscle system actually.

So, increase in creatine kinase in cerebrospinal fluid has been associated with the number of the disorders in dogs, cat, cattle's and horses. The creatine kinase are much sensitive indicator of muscle damage that generally only large increase in serum activity of a clinical significance. Normal value is in the 10 to 50 international unit per ml.

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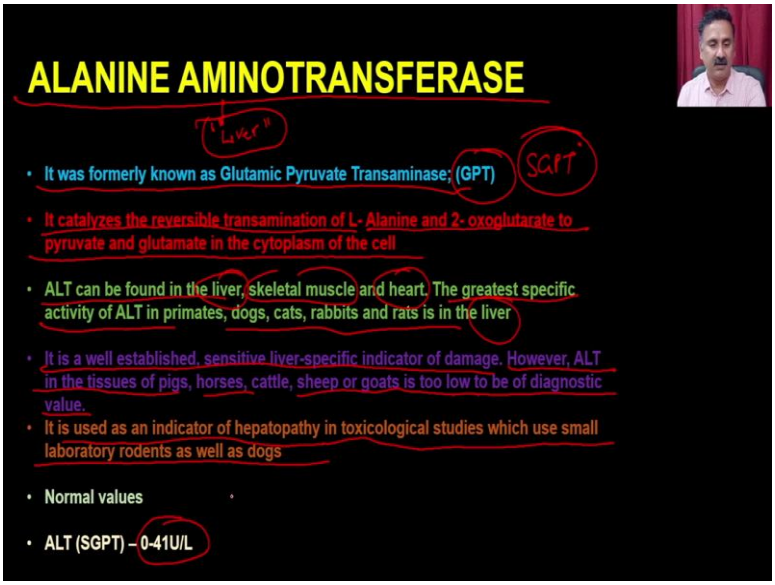

Then we have the Alanine Aminotransferases or AlT right. It was formerly known as the glutamic pyruvate transferases or the GPT or SGPT ok. If it is present in serum, then it is going to be called as SGPT. It catalyzes the reversible transamination of the l alanine and 2 oxoglutarate to pyruvate and glutamate in the cytoplasm of the cell.

ALT can be found in the liver, skeleton muscles and heart. The latest specific activity of ALT in the primates, dogs and cats are in the liver. So, it is a liver specific enzyme and it is actually going to be very specific in the liver. It is well established sensitive liver specific indicator of the damage.

However, ALT in the tissue of tissues of the pig, horse, cattle, sheep or goat is too low to be of diagnostic values. It is used as an indicator of hepatopathy in toxicological studies which use the small laboratory rodent and as well as the dogs. The normal level of the SGPT or ALT is that 0 to 41 international units per deciliter.

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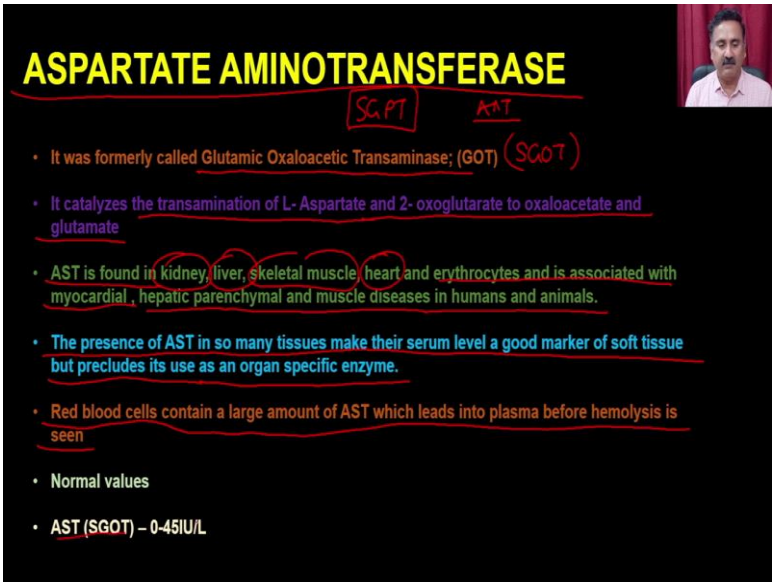

Then we have the Aspartate Aminotransferase or AAT, right. AAT is also called as the glutamate oxaloacetate transaminase or GOT or also called as SGOT ok. So, that is SG-PT. This is SGOT ok and both of these enzymes are being transiminases. So, it catalyzes the transimination of the l aspartate and 2 oxaloacetate to convert the oxaloacetate and glutamate.

AST is found in kidney, liver, skeleton muscles, heart and erthrocytes and it is associated with the myocardial hepatic, parenchymal and muscle diseases in the human and animals. The presence of AST in many tissues make their serum level a good marker of soft tissues, but precludes it use as a organ specific enzyme.

Since the AST is present in the many types of different organs, it is specificity is not being used to detect the damage in a particular organ. Red blood cell contains a large amount of AST which leads to the plasma before hemolysis is seen, normal level of AST is in the 0 to 45 interstitial units.

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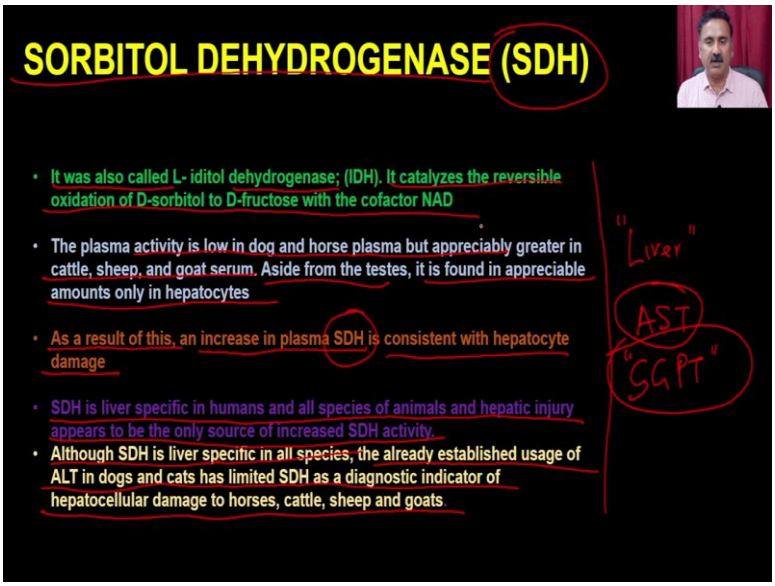

Then we have the sorbitol dehydrogenase or SDH. So, sorbitol dehydrogenase it is also called as L-iditol dehydrogenase or IDH. It catalyzes the reversible oxidation of D sorbital to D - fructose with the help of a cofactor which is called NAD. The plasma activity is low and the in dog and horse plasma, but appreciably greater in a cattle, sheeps and goat serum.

Aside from the testes, it is found in the appreciable amount only into the hepatocyte. As a result of this, an increase in plasma, sorbitol dehydrogenase is consistent with the hepatocyte damage. SDH is a liver specific in human and all species of animal and hepatic injury appear to be only source of increased SDH activity.

Although the SDH is liver specific in all species, the already established usage of ALT in dog and cat has limited the SDH as a diagnostic indicator of the hepatocellular damage to horse cattle and sheep. So, SDH is a liver specific enzyme, but since we already have the AST or SGOT right sorry SGPT as a very specific enzyme, people are not very interested to use the sorbitol dehydrogenase as a marker for detecting the damage into the liver.

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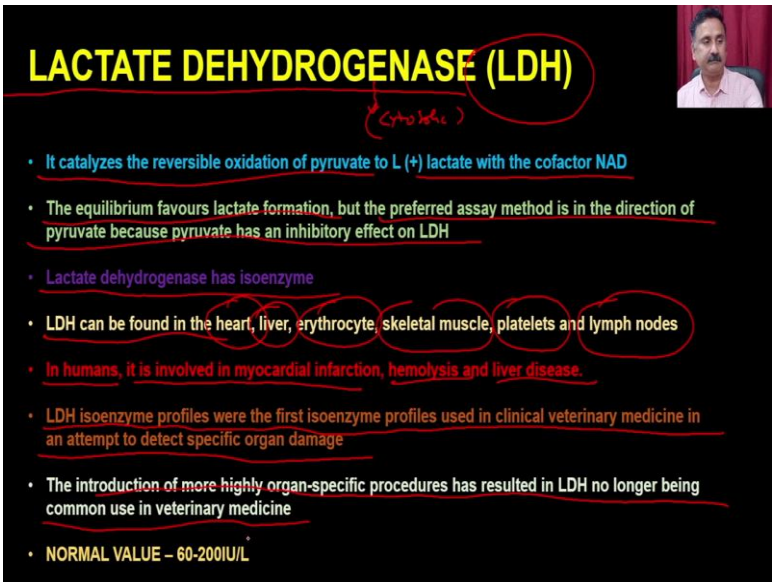

Then we have the lactate dehydrogenase or LDH. So, remember that LDH is a cytosolic marker. So, LDH is present in the cytosol of the cell. It catalyzes the reversible oxidation of pyruvate to lactate with the cofactor NAD plus. The equilibrium favors lactate formation, but the preferred assay method is in the direction of the pyruvate because the pyruvate has an inhibitory effect on LDH.

Lactate dehydrogenase has the isoenzymes. So, it can have the different types of isoenzymes and LDH can be found in the heart, can be found in liver, erythrocytes, skeleton muscles, platelets and the lymph nodes. In human, it is involved in the detection of the myocardial infarctions, hemolysis and the liver diseases. LDH isoenzymes profile were the first isoenzyme profile used in the clinical veterinary medicine in an attempt to detect the specific organ damage.

The introduction of the moral more highly organ specific procedure has resulted in the LDH no longer being used commonly in the veterinary medicines. The normal level of the LDH is 60 to 200 international units.

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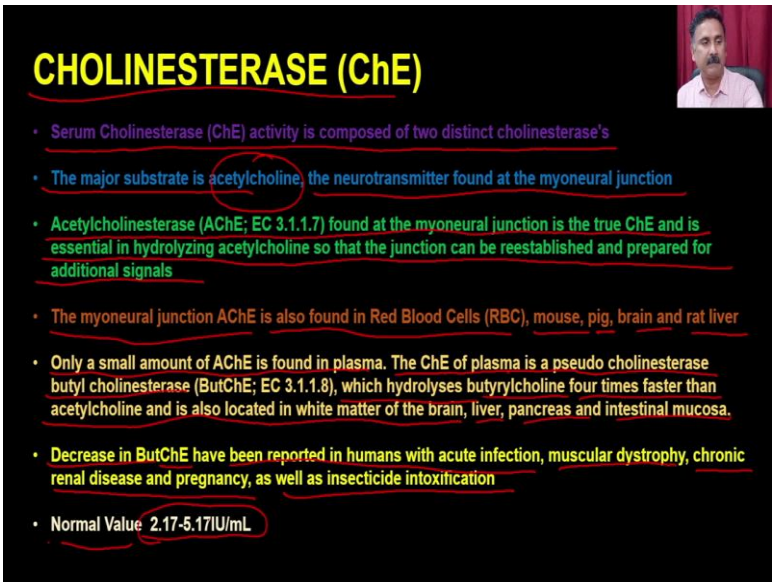

Then we have the cholinesterases, cholinesterases or ChE. So, serum cholinesterases activity is composed of 2 distinct cholinesterases. The major substrate is acetylcholine, the neurotransmitter found at the myoneural junctions. Acetylcholinesterases found at the myoneural junction is a true cholinesterases and it is essential in the hydrolyzing acetylcholine. So, that the junction can be reestablished and prepared for the additional signal.

The myoneural junction acetylcholinesterase is also found in the red blood cells, mouse, pig brain and rat livers. Only a small amount of acetylcholinesterase is found in the plasma. The acetylcholine the cholinesterase is of plasma is a pseudo cholinesterase, butyl cholinesterase which hydrolyzss the butyrylcholine 4 times faster than the acetylcholine and it is also located in the white matter of the brain, liver, pancreas, and intestinal mucosa.

Degrees in the butyryl cholinesterase have been reported in the human with the acute infection, muscular dystrophy, chronic renal diseases and pregnancy and as well as the insecticide intoxifications. The normal value of cholinesterases in the range is are in the range of 2.25 international units per deciliter.

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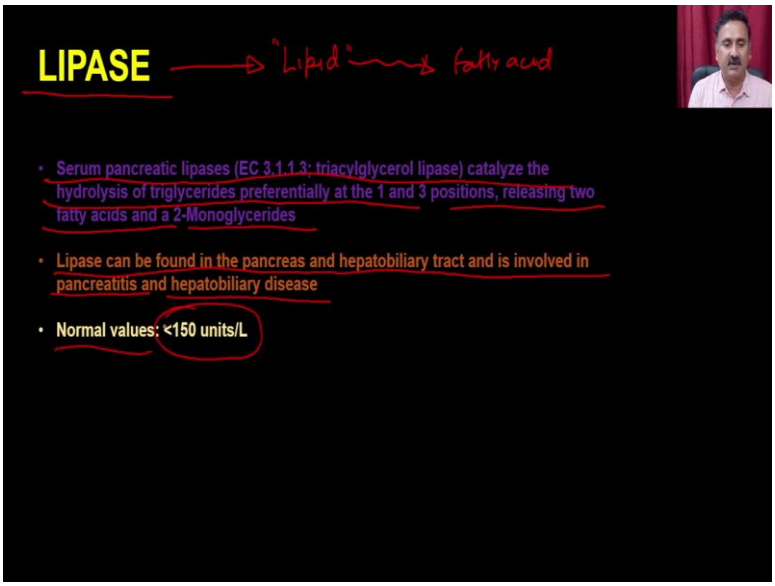

Then we have the Lipases. So, lipases as the name suggest it is actually going to use for the degradation of lipids right and that is how it is actually going to produce the fatty acid. So, serum pancreatic lipase catalyzes the hydrolysis of triglycerides preferentially at the 1 and 3 position releasing the 2 fatty acid and the monoglycerides lipase can be found in pancreas and hepatobiliary tract and it is involved in the pancreatitis and hepatobiliary disease. Normal levels are with less than 150 units per deciliter.

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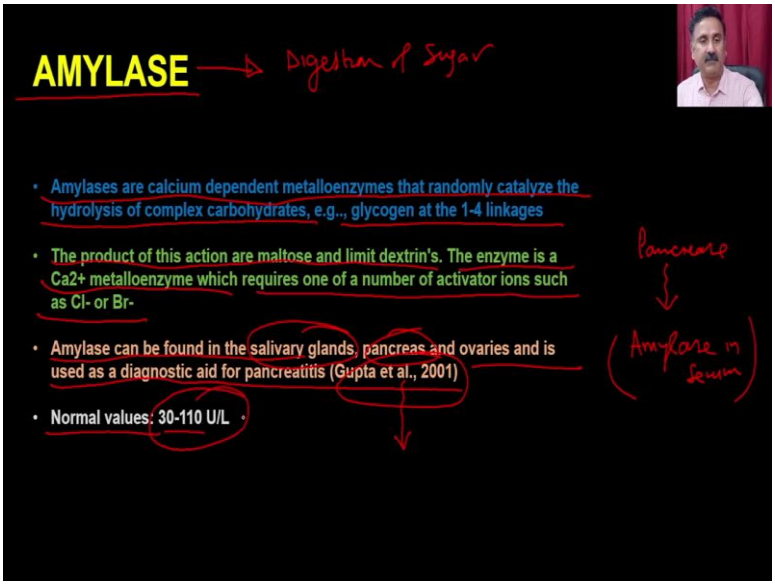

Then we have the Amylase, amylase is a enzyme what is present in the our saliva and it is actually being used for digestion of the sugar right, the digestion of the sugar. So, amylases are calcium dependent metalloenzyme that randomly catalyzes the dehydrolysis of complex carbohydrate glycogen at the 1 to 4 linkages.

The product of this action are maltose and limit dextrin. The enzyme is a calcium dependent metalloenzyme, which require one of the number of activator ions such as chloride or bromide.

Amylase can be found into the salivary glands, pancreas and the ovaries and it is used as a diagnostic aid for the pancreatitis ok. So, if you want to read more about this you can actually be able to download this particular reference and you can be able to get the more detail about how you can be able to use the amylase for detecting the pancreatitis.

So, since the amylase is present in a very large quantity within the pancreas. So, if there will be any damage to the pancreatitis it is actually going to release the amylase in serum right and that is how you can actually be able to detect the amylase. The normal level are in the range of 32-110 units per deciliter.

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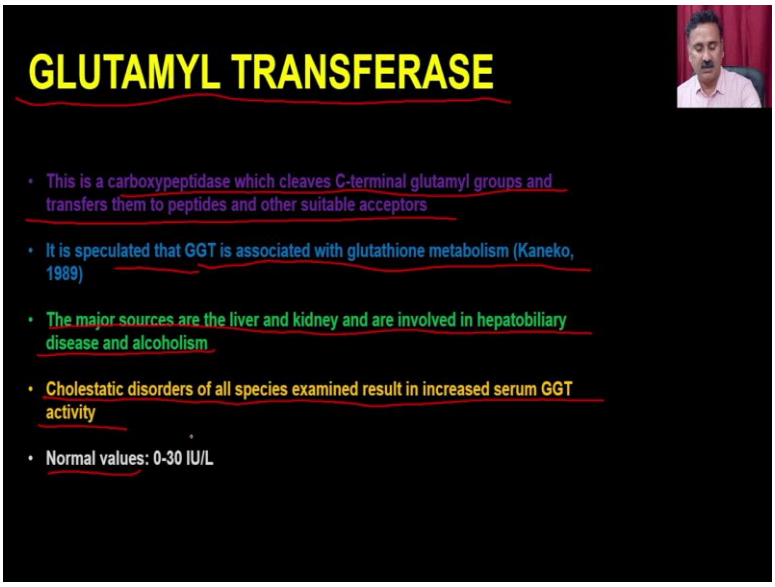

Then we have another enzyme which is called as the Glutamyl Transferases. So, this is the carboxy peptidase, which cleaves the C-terminal glutamyl group and transfer them to

the peptides and other suitable acceptor. It is speculated that the glutamyl gamma transferase is associated with the glutathione metabolisms.

The major source are the liver and kidney are it involved in the hepatobiliary disease and alcoholisms cholestatic disorder of all species examined result in the increased level of GGT activities. The normal levels are in the range of 0 to 30 international units per deciliter.

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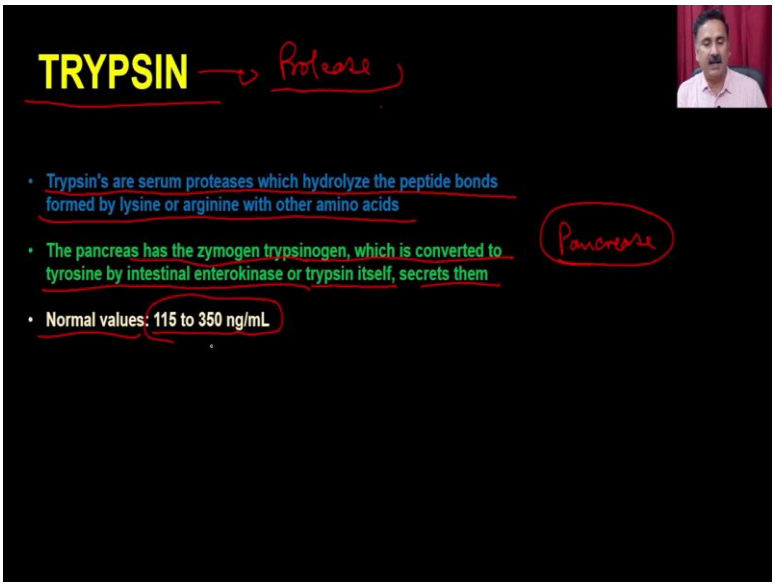

Then we have a Trypsin, trypsin is a protease right and the protease are actually going to be used for. So, trypsin's are the serum protease which hydrolyze peptide bond formed by the lysine or arginine with the other amino acid. The pancreas has the zymogen trypsinogen which is converted to the trypsin by the internal enterokinase or trypsin itself, and secret them.

And since it is present in the pancreas it is actually going to give you the detection of the pancreas damage. Normal level are in the range of 115 to 350 nano grams per ml and its actually going to use. So, these are the some of the enzymes, but you can actually be used for diagnostic purpose. Apart from this the enzymes can also be able to use to perform the different types of assays and these assays are also being used for diagnostic purposes.

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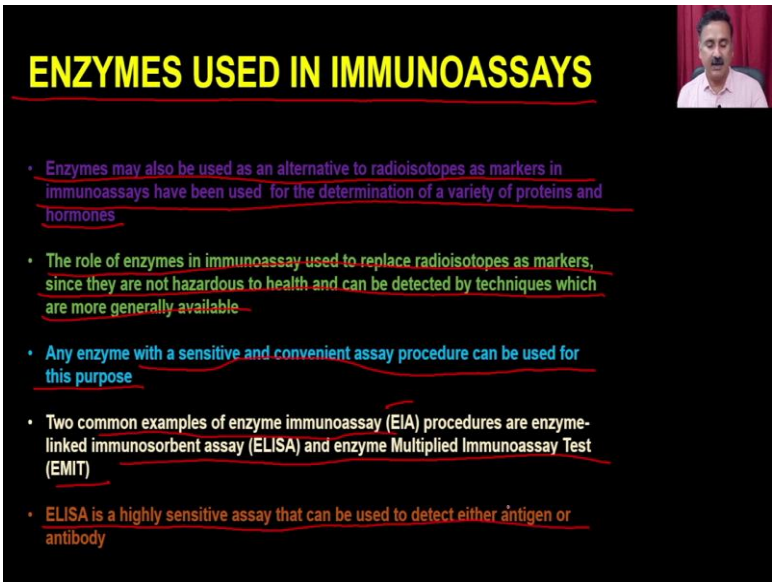

So, enzymes are being used in the immuno assays. For example, enzyme may also be used as an alternative to radioisotope as marker in the immuno assay and has been used for the determination of variety of proteins and hormones. The role of enzyme in the immuno assays used to replace radioisotope as markers. Since they had not hazardous to the health and can be detected by techniques which are more generally available.

Any enzyme with the sensitive and convenient assay procedure can be used for this purpose. The 2 common example of the enzyme immuno assay are enzyme linked immunosorbent assay ELISA and enzyme multiply immuno assay test or emit. ELISA is a very highly specific technique which can be used to detect either antigen or the antibodies.

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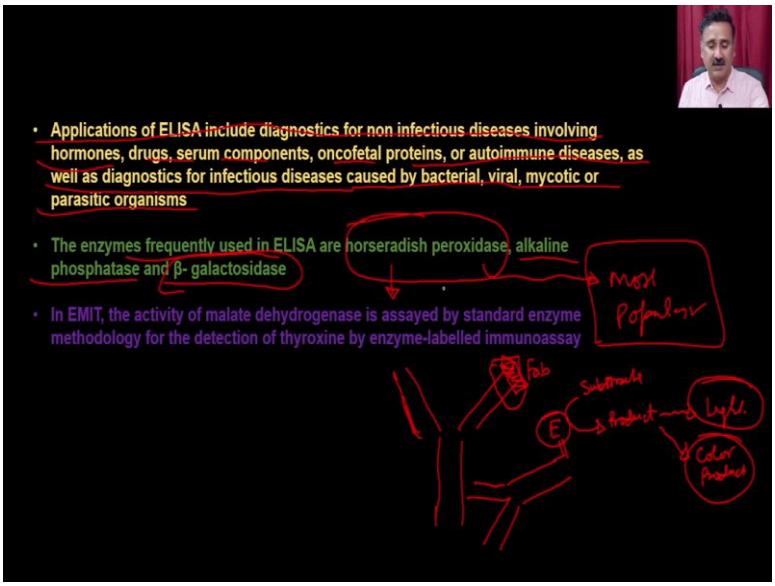

Applications of ELISA include the diagnostics for the non-infectious diseases involving the hormones, drugs, serum component, oncofetal proteins, autoimmune diseases as well as diagnostic for the infectious disease caused by the bacteria, virus, mycotic and parasitic organisms. The enzyme frequently used in ELISA are the horseradish peroxidase ok and the alkaline phosphatase and the beta galactosidease ok.

And the alkaline phosphatase is the most popular enzyme what you are actually going to use in the ELISA ok. And so if you remember that the enzyme if you remember the structure of the antibody right. So, ELISA in ELISA you are going to use the 2 different types of antibodies.

You are going to use the primary antibody which is actually going to be used for detection of the antigen and then you are going to use the secondary antibody which is actually being coupled with the enzyme. So, this is the you know the primary antibody. So, in the primary antibody you are going to have the antigen binding site. So, this is the antigen binding site and this portion is called as a constant portion.

And this antigen binding site will actually go and bind to the antigen. So, for example, if this is the antigen, so if this is the antigen it is actually going to bind the antigen and then it is actually going to release the signal. But on the back side it is actually going to be detected by the secondary antibodies right. So, secondary antibodies are actually being coupled with the enzyme.

And the most popular enzyme in this case is the horse-radish peroxidase. So, when this is actually going to detect this particular enzyme is actually going to convert the substrate into a product. And this mostly this product are either going to give you the light or it is actually going to give you a colored product. And by that you can actually be able to detect ok.

Some cases sometimes what happens is that product is actually going to give you the precipitate or sometime in this product is going to give you a colored product or sometime it is actually going to give you a light and all these can be detected using the different types of spectroscopic methods. So, let us discuss little bit about how you can be able to use the horse-radish peroxidase and how you can be able to reduce the peroxidase in the for the these kind of applications.

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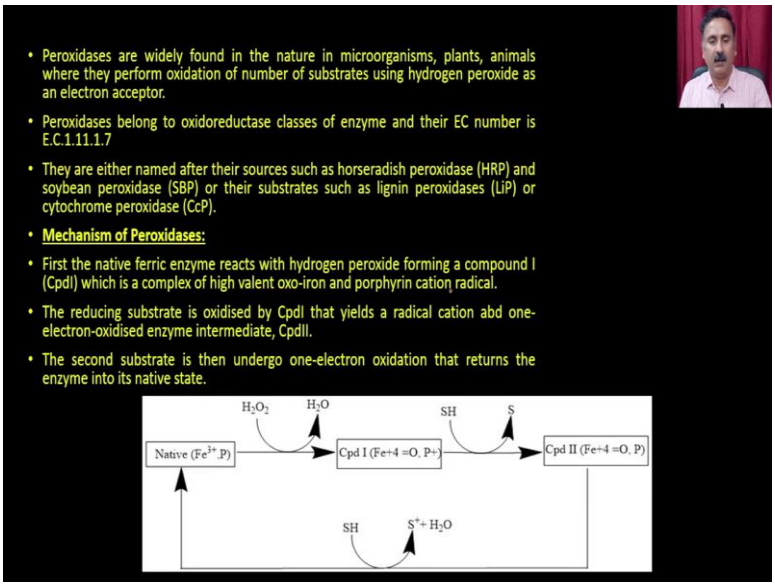

So, peroxidases are widely found in the nature in the microorganisms, plants, animals where they perform the oxidation of number of substrate using the hydrogen peroxide as an electron acceptors. Peroxidase belongs to the oxidoreductase class of enzyme and their EC number is 1.11.1.7 ok.

They are either named after their source such as horse-radish peroxidase and soybean peroxidase or their substrate such as lignin peroxidase or the cytochrome c peroxidase. Mechanism of peroxidase activity first the native descend all you can actually be able to reach. So, this is what it is actually going to follow a reaction mechanism and that is how

it is actually going to oxidize the product and that oxidation product could polymerize with each other, because it is actually going to generate the single electrons and that is how it is actually going to form the polymer of the product.

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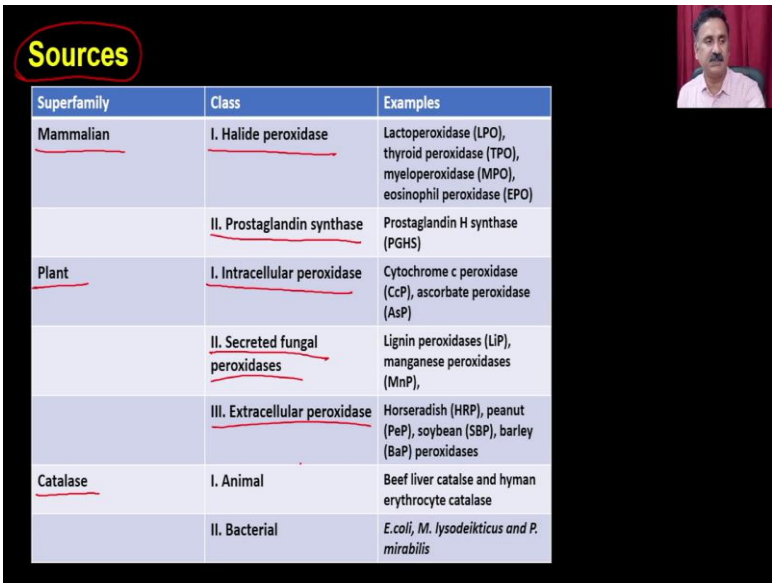

And that is how the polymer of that product is actually going to give you the color. As far as the sources of the peroxidase is concerned it can be from the mammalian sources, plant sources or catalase. So, you can have the halide peroxidase, you can have the prostaglandin synthase you can have intracellular peroxidase, you can have secreted fungal peroxidase, extracellular peroxidase and so on. Similarly, you can have the catalase, which is going to be from animal or bacterial sources.

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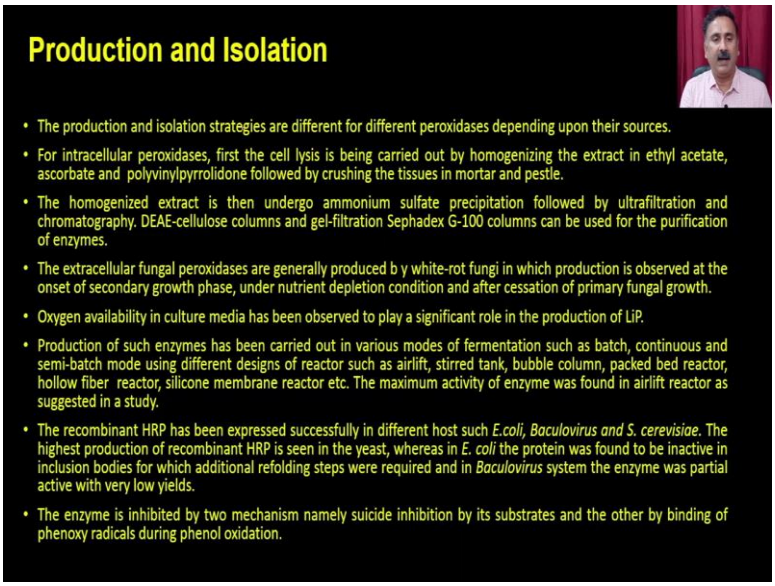

And as far as the production and isolation is concerned the production and isolation strategies are different from different peroxidases depending upon their sources. For intercellular peroxidases for the cell lysis is being carried out by the homogenization, the extract and the extract is going to be present in the ethyl acetate, ascorbate and PVD followed by crushing the tissue in morter and model.

So, these are the different you know full scheme what you are going to use for production and isolations and then it is actually going to use for different types of applications.

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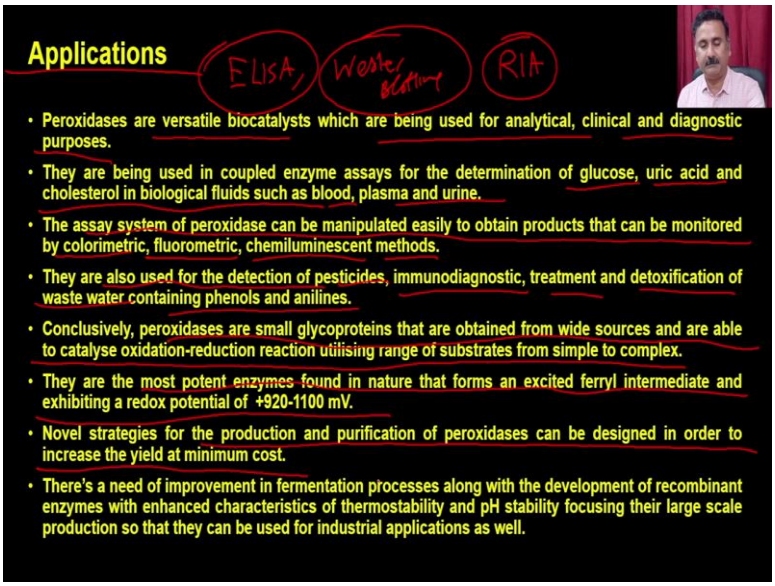

So, peroxidases are versatile biocatalysts which are being used for the analytical, clinical and diagnostic purposes. Remember that the peroxidase are being used in ELISA, it is going to be used in western blotting and other kinds of blotting technique as well right. And it is also going to be used in RIA and all of the kinds of technological techniques what you are going to use.

They are being used in coupled enzyme assay for the determination of glucose, uric acids, cholesterol in the biological fluids such as blood, plasma and urine. The assay system of the peroxidase can be manipulated easily to obtain the product that can be monitored by the c colorimetric method, fluorometric method, chemiluminescent method and so on.

They are also being used for the detection of pesticides, immunodiagnostics, treatments and detoxification of the wastewater containing the phenols and anilines, because what they are going to do is they are going to oxidize these phenolines or the anilines and once they get oxidized, they will actually going to form the product.

You know the adapt and once the these toxic products are actually going to form the adapt then activity is going to be somehow reduced or they are actually going to be isolated from the system. Peroxides peroxidases are small glycoprotein that are obtained from the wide sources and able to catalyze the oxidation reaction involving utilizing range of substrate from simple to complex. They are most protein enzyme found in

nature and forms an excited, ferryl intermediate and exhibiting a redox potential of 920 to 1110 millivolts.

Novel strategies for the production and purification of peroxidase can be designed in order to increase the yield at a minimal cost and there is a need to in the improvement of fermentation process, so that you can be able to produce the peroxidases in the large quantities.

So, this is all about the application of enzyme into the food industry and very briefly we have also discussed about the application of enzyme into the medical field. In our subsequent lecture we are going to discuss some more aspects of the enzyme application in the other fields. So, with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss some more aspect related to the enzyme science and technology.

Thank you.