Manage TB
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Lecture – 13
Molecular Diagnosis of Tuberculosis
Session 01

Hello every one; welcome to the session on Molecular Diagnosis of Tuberculosis. I am Dr. Uma Devi scientist the national institute for research in tuberculosis Chennai.

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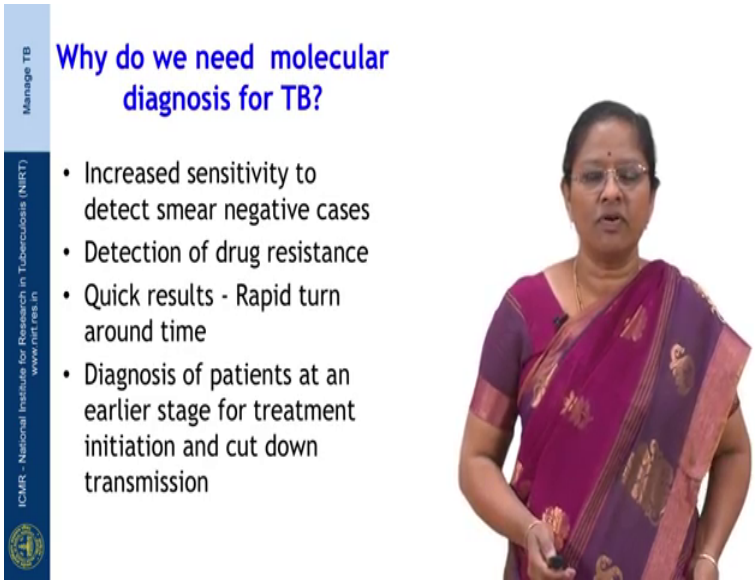

First of all let us understand why do we need a molecular diagnosis for tuberculosis? As we all know earlier accurate diagnosis of tuberculosis is essential for effective management of the patients and there are many methodologies that are available for diagnosis of tuberculosis. In the earlier sessions you must have heard about the convention methods of diagnosis including smear microscopy as well as culture and drug susceptibility testing by phenotypic methods.

And as we all know a smear microscopy has its own sensitivity because we required about 10000 bacilli per ml of the sportive for deducting positivity and culture is a time taking process. So, the advantage of a using a molecular diagnosis is that; it has a rapid speed of deduction for turnaround of the results. So, if we look at molecular diagnosis it has a increased sensitivity to detect smear negative cases and it can detect also the drug

resistance in addition to the diagnosis, as well as the results have a quick turnaround time varying between 2 hours to 48 hours depending on the different methodologies.

Therefore this method can help in the diagnosis of patients at an earlier stage for treatment initiation and to cut down transmission.

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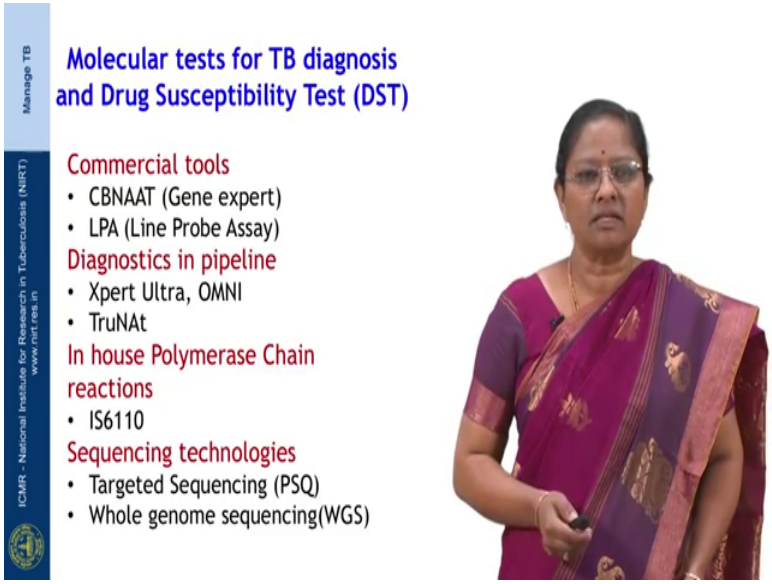

So, the next slide I am going to show about the different molecular tests that are currently available for doing diagnosis of tuberculosis and drug susceptibility testing. 1 is the commercial tools that are currently in place which includes the cb-naat and one of the methods used by GeneXpert and the other is line probe assay; following that I will also be discussing about the diagnostics that are in pipeline which includes the expert ultra, the OMNI and the TruNAts devices.

And there are also in-house polymerase chain reactions which I will be touching on during my presentation. I will also be touching on few of the sequencing technologies like; the targeted sequencing and the whole genome sequencing; which is currently not utilised for patient managements in India, but which can be utilised for discordant resource or when there is further confirmation required.

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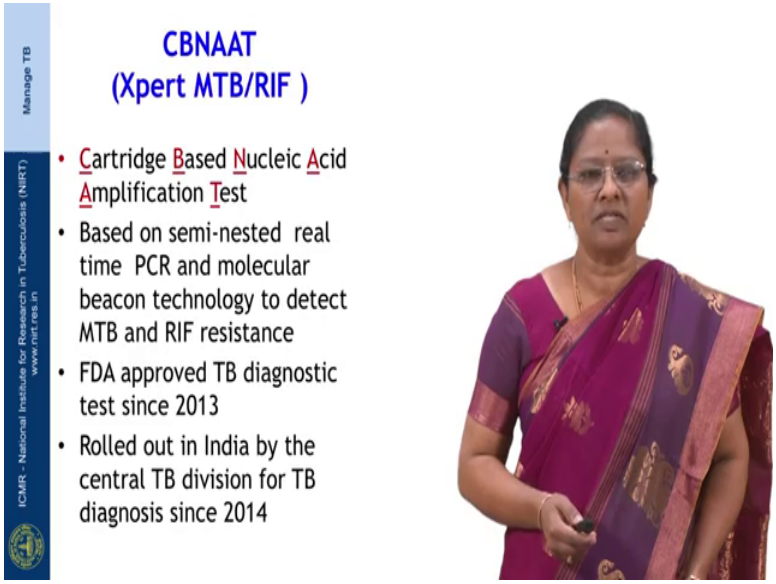

Coming to cb-naat or the cartridge based nucleic acid amplification test; currently we have the expert MTB RIF which was one of the advanced technologies that had made a trend setting in the diagnosis of tuberculosis and rifampicin resistance reduction. This technology is based on a semi nested real time PCR and molecular beacon technology to detect MTB as well as rifampicin resistance.

So, this test has been approved by the US FDA as a diagnostic test for TB since 2013. So, it has been rolled out in India by the central TB division for TB diagnosis since 2014.

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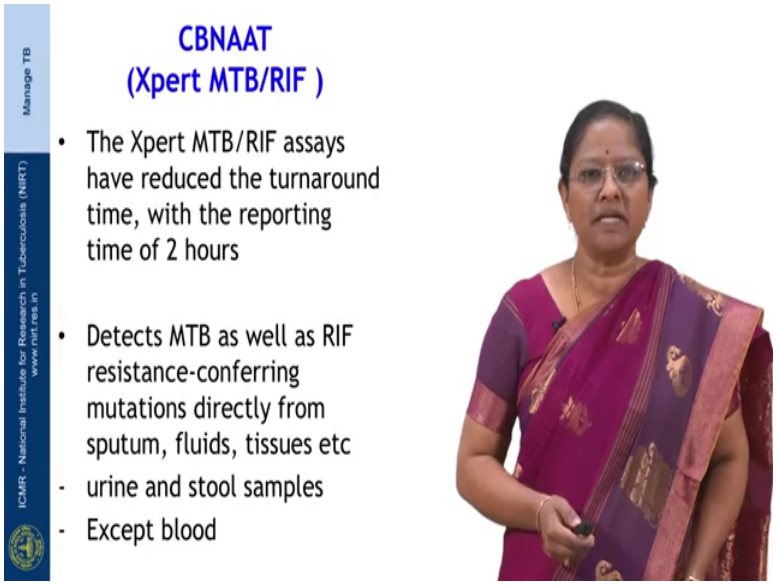

The expert MTB RIF assays has reduced the turnaround time for deduction with the reporting time of 2 hours compared to the conventional methodologies which uses which requires more than a few weeks for deduction and drug resistance reporting.

In addition to deducting M tuberculosis it also detects rifampicin resistance mutations and this can be utilized for detection of TB and RIF resistance in biological specimens like sputum, fluids and tissues. There are being a considerable amount of evidence that has been produced for rifampicin reduction and diagnosis of using expert in urine as well as stool samples. However, blood could not be an ideal specimen for diagnosis using GeneXpert. Currently the program recommends the deduction of sputum, fluids and tissues using GeneXpert.

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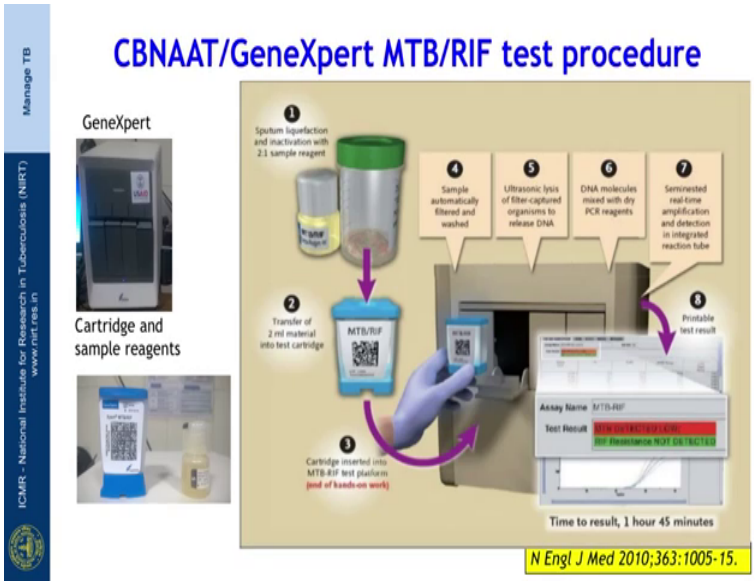

This cartoon shows the actual procedure that is followed for GeneXpert deduction. On the left side you can see the GeneXpert mission this is a 4 module cartridge that is used here, there are different machines which uses either a 1 module system, 4 modular or a 16 module system.

And the bottom on the left you can see the cartridge that is being put into this modules and the lysis buffer that is provided along with this cartridge. So, as such the hands on required during this procedure is only for 2 minutes duration during which the sample is added to the container which is given and then the buffer is added to sample in the

container in the ratio of 2 is to 1 ratio and then left for incubation for 15 minutes at room temperature.

Later 2 ml of this material is transferred into a test cartridge with this the hands on work on this assay is completed this close this cartridges is then placed into the module of the expert mission and where the sample is actually filtered, washed and the DNA is released and the DNA is also mixed with the PCR reagents and then following by following this there is a semi nested real time application which occurs this mission and the results are seen through a desktop or a laptop screen and which is a printable results that is obtained.

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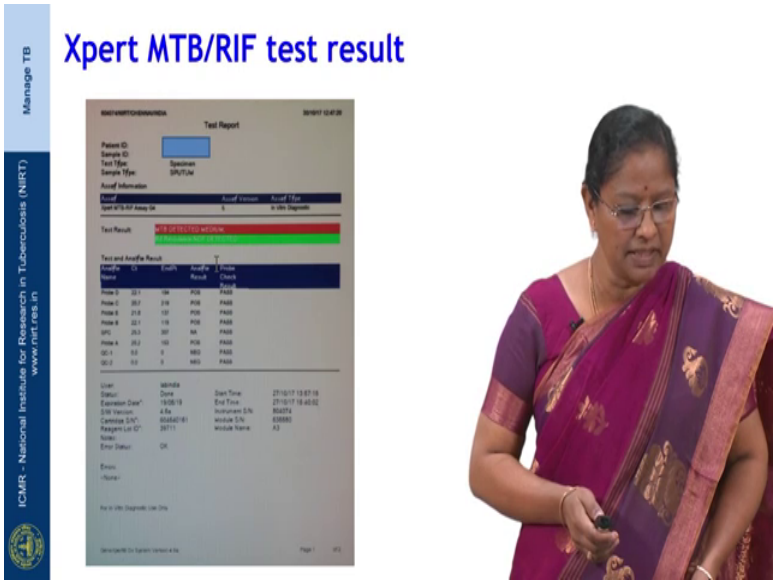

This slide shows the patient id and the sampled number and also gives the test results other as to whether the biological sample is MTB detected MTB detector or not detected and also resistance deduction for riffampicin or absence of resistance for rifampicin. At the bottom it also gives the results for each of the 5 proofs that are used in the assay and also it has a sputum processing control.

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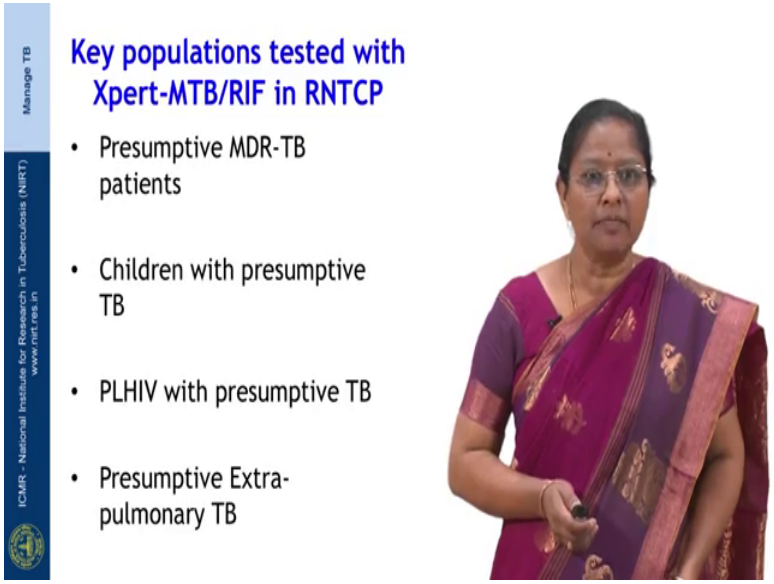

So, let us look at the key population that are tested by MTB RIF as given by the guidelines of RNTCP. So, in patients who are suspected of MDR tuberculosis which includes retreatment cases, failure cases or contacts of MDR TB could be tested for GeneXpert and also childrens who was suspected for tuberculosis and people who are living with HIV and having symptoms suggestive of tuberculosis could be tested by GeneXpert.

And also GeneXpert is useful for deduction of a the tuberculosis extra pulmonary patients the case of extra pulmonary samples; that like biopsies which requires additional processing compared to regular biological specimen like sputum, but the assay time on the mission is the same as biological specimens.

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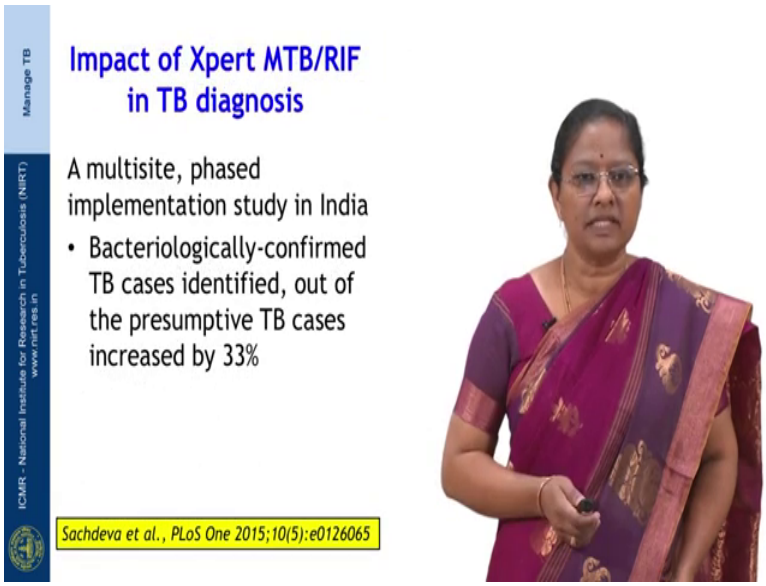

When the GeneXpert was initially rolled out in the country it was faced it was in a faced manner and a multisite faced implementation study was carried out in India, where you have shown that there was a 33 percent increase in present TB case deduction after the rolling out of GeneXpert such was the power of this technology.

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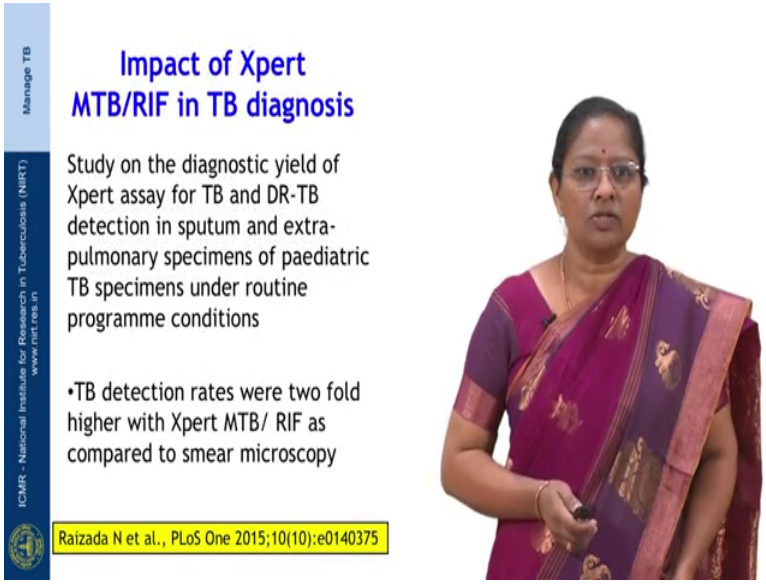

And also there was a study which looked into the paediatric TB population for the diagnosis of tuberculosis and drug resistance deduction in the sputum as well as in the

extra pulmonary specimens; where again TB deduction rate was found to fold higher with expert MTB RIF as compared to smear microscopy.

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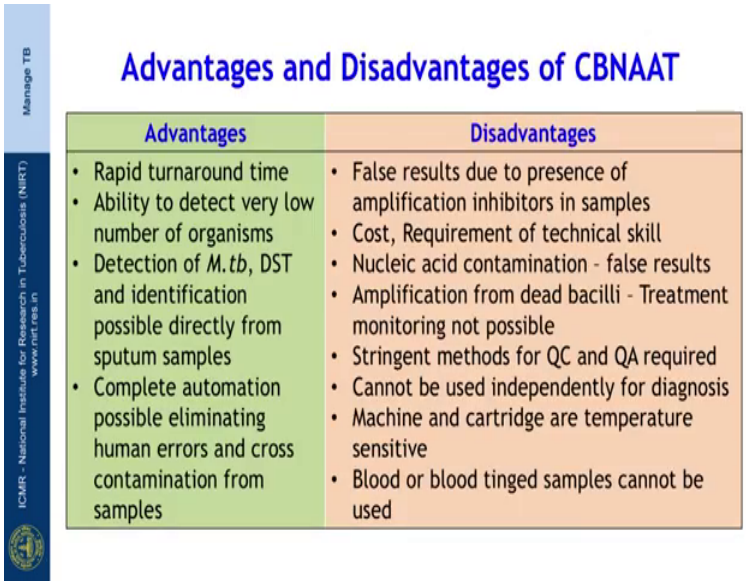

So, if you look at the advantages and disadvantages of using cb-naat; the advantage is that it has a rapid turnaround time and it has the ability to detect very low number of organisms and the deduction of MTB along with drug susceptibility testing could be directly done from the sputum samples. And it is a completely automated process except for the 2 minutes hands on that is required in the initial stages and it eliminates any human error and cross contamination from the samples because it is a closed system.

The disadvantages of using that cb-naat is that it could lead to false positive results due to the presence of amplification inhibition in the samples and there are chances that it might have nucleus is contamination which might lead to faults results; if the assay is not done carefully.

The most important thing is the DNA in the dead bacilli could be amplified using the cb-naat therefore, it could not it should not be advice for patients who are in treatment. Stringent methods for quality control and quality assurance is required for performing this assay in the laboratory and it cannot be used independently for the diagnosis and it can be confirmed with phenotypic DST in discordant results.

The machine and the cartridge for GeneXpert is temperature sensitive and it require special specialized speciality. Care should be taken to make sure that blood and blood samples on its not sent to the laboratory for diagnosis using cb-naat.

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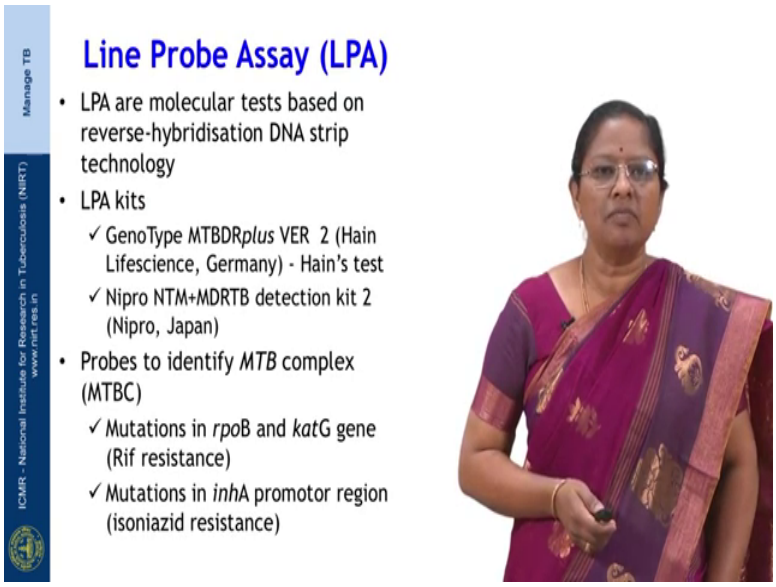

So, next available technology is the line probe assay; the line probe assay is also a molecule test which is based on a reverse hybridization DNA strip technology.

There are two kits of NPA which has been approved by FDA. One is the genotype MTBDR plus version 2; which is the Hain's lifescience kit which is for which is generally called Hain test. The other one is the Nipro NTM MDR TB detection kit 2; when the former deducts MTB and resistor to drug resistance the later kit detects MTB and drug resistance along with the few of the non tuberculous mycobacterial species identification.

So, the probes that are usually used in these kits are for the deduction of mutations in the rpoB and the katG region for rifampicin resistance and for mutations for inhA promoter for isoniazid rifampicin isoniazid resistance.

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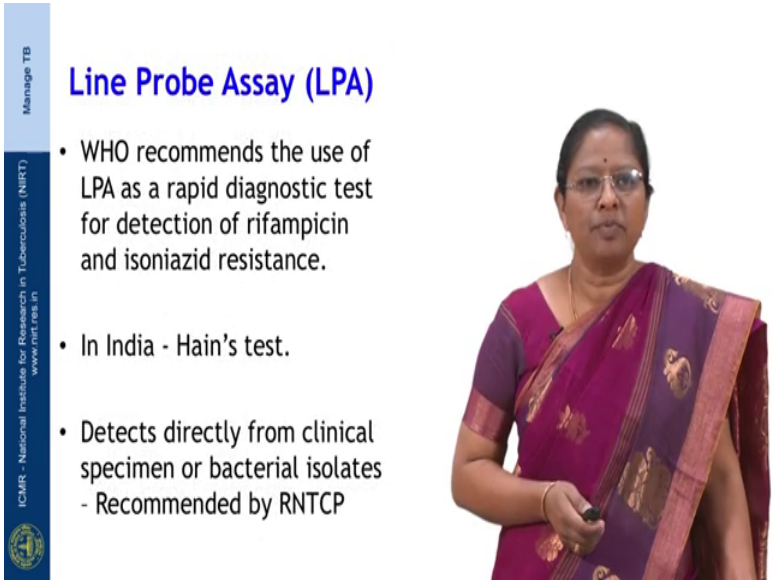

So, the line probe assay was recommended by WHO for use as rapid diagnostic test for the detection of rifampicin as well as isoniazid resistance. In India under the programmatic settings it is the Hain test is more commonly used and it detects directly from the clinical specimen when you call it is a direct testing and also from the bacterial isolates in case if this smear is negative.

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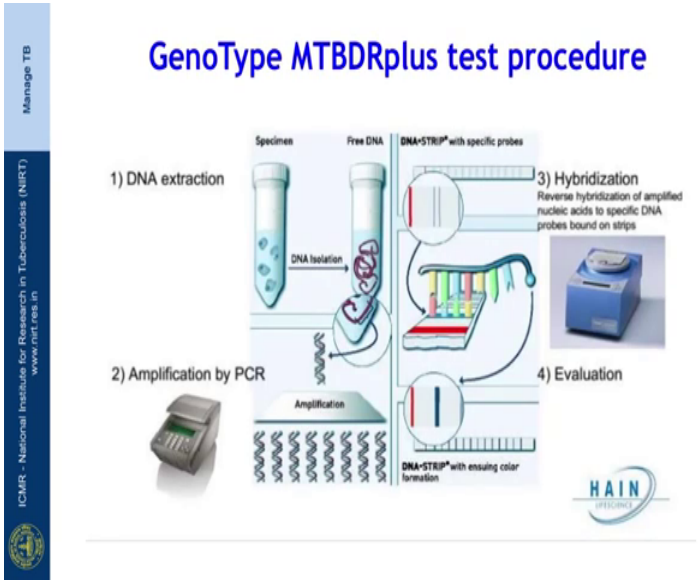

This cartoon gives the overview of the genotype mdb MTBDR plus test procedure.

So, there are 4 major steps involved here one is the DNA extraction from the bacilli obtained from the smear positive specimens, followed by the release of the DNA from these smear specimens. The second step is the amplification by PCR using specific probes and the third steps involves the reverse hybridization procedure; where the amplified nuclic acid is bound to a DNA probes on the strips.

The third step is a hybridization procedure, which involves the reverse hybridization of amplified nucleic acid to specific DNA probes that are bound on the strips and following by that is evaluation which is based on enzyme conjugate reaction and development of bands on the strips.

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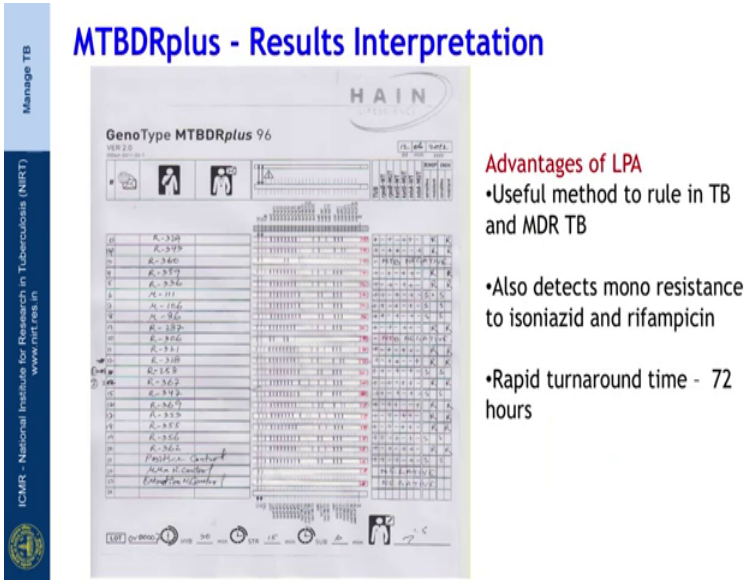

So, this slides gives the overview of the result that is generated after doing a line probe assay. As you can see each trip has about 27 loci is located on it which is basically it has a controlled for the conjugates amplification controls and also a bad for the tuberculosis diagnosis. Apart from that it has loci for rpoB, katG and inhA and in addition it has a wild type loci and the mutant loci for all the three genes that are the rpoB, katG and inhA respectively. Based on the presence or absence of genes in each of these loci the pattern of resistance and the identification of MTB is decided.

So, if you can look at the right side of the result sheet you can see on the first line you can see that; it is positive it has a band positive for tuberculosis and it is negative for wild type rpoB and it is positive on mutation for katG resistance and so this strain is resistant

to rifampicin and isoniazid and it is an MTB. So, the third line shows that it is negative for the tuberculosis band and therefore, it is a MTB negative strain. So, this LPA is usually a useful methodology to rule in tuberculosis and multidrug resistant tuberculosis.

So, this can also be used to detect mono resistance isoniazid as well as rifampicin and the turnaround time for LPA results is about 72 hours if the deduction is made directly in the sputum. But in the case of smear negative cases it may take more than 48 hours to 1 week.

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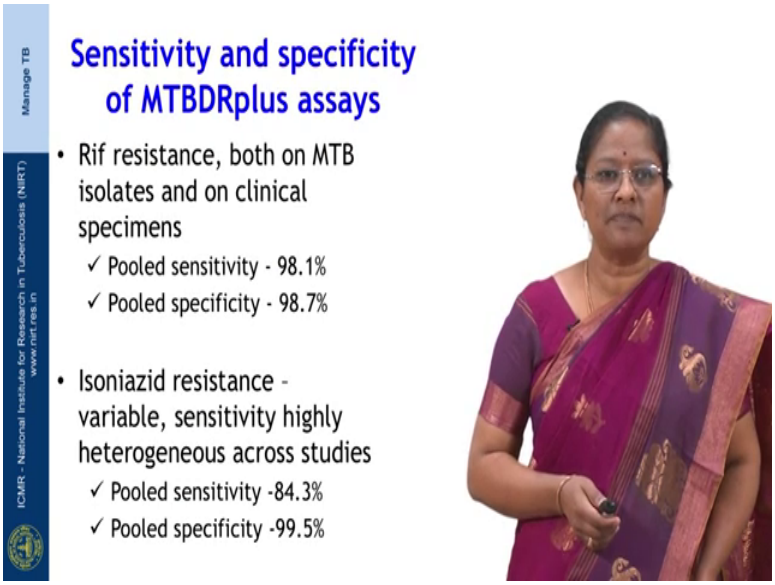

If you look at the specificity and sensitivity of MTBDR plus assays; rifampician resistance both on MTB isolates and or the clinical specimens it is has the pooled sensitivity of 98 percent and pooled specificity of 98 percent 98.7 percent.

For isoniazid resistance reduction it has variable sensitivity and it is heterogeneous across different studies. So, if we look at the pooled sensitivity is about 84.3 percent sensitive for isoniazid resistance deduction and pools specificity of 99.5 percent.

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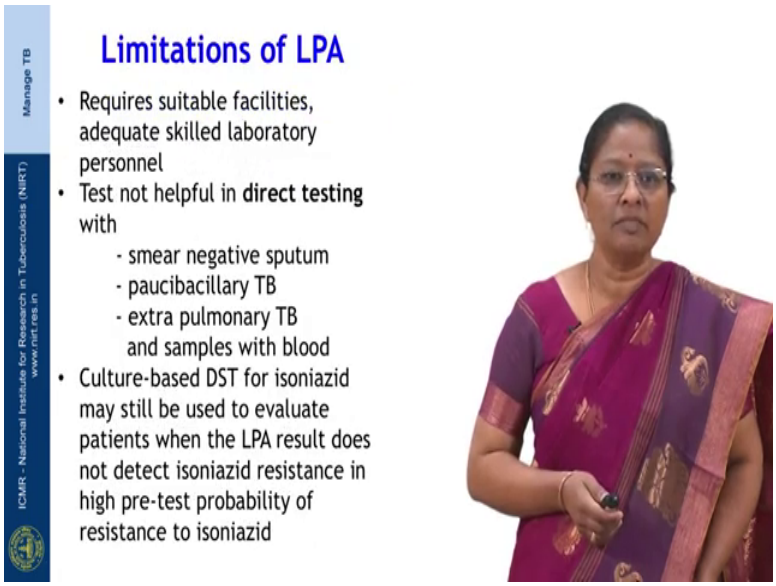

What are the limitations of the line probe assay? The line probe assay requires a suitable laboratory facility with the adequate skilled laboratory personnel and the test is not useful in direct testing with smear negative sputum, the paucibacillary TB cases, in the extra pulmonary TB as well as in samples with blood.

So, culture based diagnosis for isoniazid resistance is still be used for evaluating patients when the LPA results are not detecting isoniazid isoniazid resistance in a high pre test is possibility of resistance to isoniazid.

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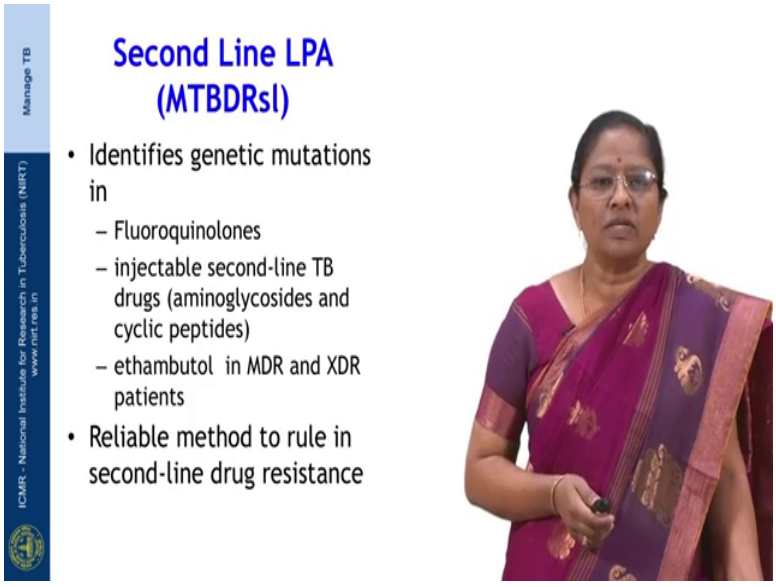

So, coming to the second line line probe assay which is called the MTBDRSL this has been recently approved by FDA and it identifies genetic mutations in the fluoroquinolone; the injectable second line TB drug namely with, aminoglycosides and cyclic peptides and also for ethambutol in MDR and XDR patients. This is again one of the reliable methods to rule in second line drug resistance.

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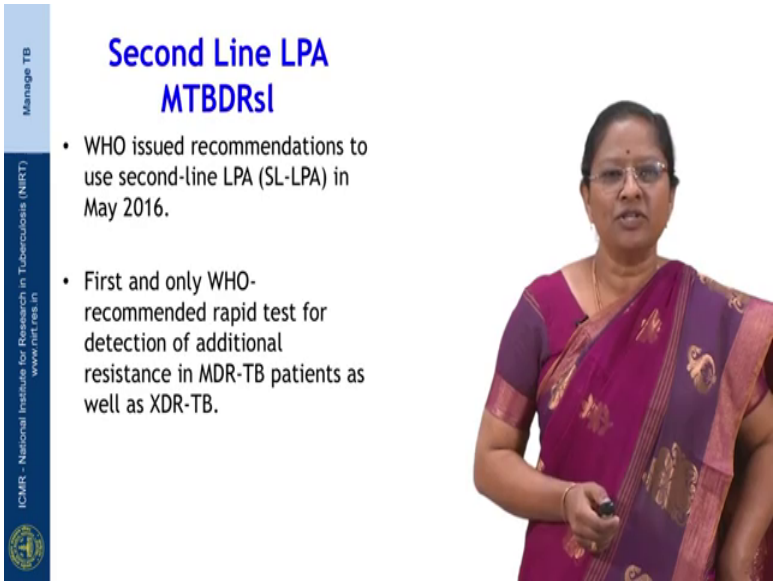

WHO issued recommendations to use the second line LPA in May 2016; this is the first and only WHO recommended rapid test for the detection of additional resistance to MDR TB patients as well as XDR TB.