Testing Covid-19 - RT-PCR and Antibody tests

What is the issue?

- The role of 'silent spreaders' in the coronavirus pandemic highlights the significance of testing. Click here to know more on this
- In this backdrop, here is a look at the two types of tests to detect the virus infection - RT-PCR based and the antibody based.

How does the Coronavirus express itself?

- A virus is a microscopic package of genetic material (either DNA or RNA) surrounded by a molecular envelope.
- Some viruses such as the coronavirus (SARS-Cov2) only contain RNA.
- This means that they rely on infiltrating healthy cells to multiply and survive.
- Once inside the cell, the virus uses its own genetic code to take control of and 'reprogramme' the cells to make them become virus-making factories.

What is the RT-PCR method in testing?

- Real time RT-PCR (reverse transcription–polymerase chain reaction) is now one of the most accurate laboratory methods for detecting, tracking, and studying the coronavirus.
- RT-PCR is a nuclear-derived method for detecting the presence of specific genetic material from any pathogen, including a virus.
- It uses markers to detect the presence targeted genetic materials.
- Originally, radioactive isotope markers were used.
- Subsequent refining has led to the replacement of the isotopic labelling with special markers, most frequently fluorescent dyes.
- With real time RT-PCR, scientists can see the results almost immediately while the process is still ongoing. [Conventional RT-PCR only provides results at the end.]

What is the principle behind?

- In order for a virus like the coronavirus to be detected early in the body, using real time RT-PCR, scientists need to convert the RNA to DNA.
- This is a process called 'reverse transcription'.
- They do this because only DNA can be copied - or amplified - which is a key part of the real time RT-PCR process for detecting viruses.

- Scientists amplify a specific part of the transcribed viral DNA hundreds of thousands of times.
- By this amplification, instead of trying to spot a minuscule amount of the virus among millions of strands of genetic information, scientists have a large enough quantity of the target sections of viral DNA.
- This facilitates in the accurate confirmation that the virus is present.

How does RT-PCR work in Coronavirus case?

- A sample is collected from parts of the body where the coronavirus gathers, such as a person's nose or throat.
- The sample is treated with several chemical solutions.
- This remove the substances, such as proteins and fats, and extracts only the RNA present in the sample.
- This extracted RNA is a mix of a person's own genetic material and, if present, the coronavirus' RNA.
- The RNA is reverse transcribed to DNA using a specific enzyme.
- Then, additional short fragments of DNA are added that are complementary to specific parts of the transcribed viral DNA.
    i. Some of the added genetic fragments are for building DNA strands during amplification.
    ii. The others are for building the DNA and adding marker labels to the strands, which are then used to detect the virus.
- These fragments attach themselves to target sections of the viral DNA if the virus is present in a sample.
- The mixture is then placed in a RT-PCR machine.
- The machine cycles through temperatures that heat and cool the mixture to trigger specific chemical reactions.
- These reactions then create new, identical copies of the target sections of viral DNA, and the cycle repeats over and over to continue copying.
- [Each cycle doubles the previous amount: two copies become four, four copies become eight, and so on.
- A standard real time RT-PCR setup usually goes through 35 cycles.
- So, by the end of the process, around 35 billion new copies of the sections of viral DNA are created from each strand of the virus present in the sample.]
- As new copies of the viral DNA sections are built, the marker labels attach to the DNA strands and then release a fluorescent dye.
- This is measured by the machine's computer and presented in real time on the screen.
- The computer tracks the amount of fluorescence in the sample after each cycle.
- When the amount goes over a certain level of fluorescence, this confirms that

the virus is present.
- Scientists also monitor how many cycles it takes to reach this level in order to estimate the severity of the infection.
- [The fewer the cycles, the more severe the viral infection is.]
- **Advantages** - This technique is highly sensitive and specific and can deliver a reliable diagnosis as fast as 3 hours, though usually laboratories take on average between 6 to 8 hours.
- Comparatively, real time RT-PCR is significantly faster and has a lower potential for contamination or errors.
- This is because the entire process can be done within a closed tube.



What is the anti-body test?

- The antibody test is based on a biological phenomenon.
- In response to entry of any ‘foreign invader’ (virus, in this case), antibodies are generated by the body's own immune system.
- Such antibodies are specific to that invader.
- Therefore, detection of specific antibodies in a blood sample may indicate that 'at some point', that individual is exposed to that invader.

What are the drawbacks in anti-body test?

- Unlike DNA, antibodies cannot be copied in a test tube, and therefore, this test is generally less sensitive than PCR.
- Also, the human body takes some time to generate antibodies after it is exposed to an invader.
- Antibodies continue to be present in the blood for some time even after the invader is completely eliminated from the body and there are no clinical symptoms.
- [The human body is capable of generating immunological memory after first infection.
- So, the next time, if same invader re-enters, the body is better prepared to eliminate the infection (a principle behind all vaccinations).
- In some cases, this memory (which is typically called “immunity”) is life long; in other cases, it is short termed.]

Why are both tests relevant now?

- Clearly, the controversy of one test over the other is misplaced; both are important, but their purposes are different.
- RT-PCR is the confirmatory test to be done when someone comes with clinical symptoms.

- The same test will certify if the patient is fully recovered or not.
- RT-PCR will give an idea if people within the immediate proximity of a newly-confirmed patient are infected by the virus.
- However, in this case, the first negative result does not completely rule out lack of infection.
- Therefore, quarantine/isolation of these people (who were in proximity with patient) is essential.
- On the other hand, the vast majority (by some estimates 70-80%) would have sub-clinical infection (they have never shown any symptoms).
- So, in this case, the antibody test will give data on how many people are exposed to the virus at some point of time.
- This would be essential to estimate the extent of virus spread in the community and to respond appropriately.

Source: The Print, IAEA

