# Title: Modified RT-LAMP assays for detection and removal of carry-over amplicon.

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#### **SUMMARY:**

Reverse transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) is an ultrasensitive tool for amplification of target nucleotide sequence. The tool is widely used in the detection of SARs-COV-2 viral genome due to its high amplification efficiency and cost-effective method. However, there is a downside associated with high-rate amplification as carry-over amplicon contaminates the solution and reagents thus jeopardizing the accuracy of the molecular diagnostic technology in clinical settings.

To address the issue, an amplicon capture system is developed where the carry-over amplicon is captured and removed using a modified RT-LAMP detection assay. The assay comprises an RT-LAMP assay mixed with highly magnetic nanoparticles of Heusler alloy. The nanoparticle is engineered using in-house designed oligonucleotide probes which contain the complementary sequence of the carry-over amplicon. The working mechanism of the assay is initiated with the attachment of the amplicon to the magnetic nanoparticle. The complex containing an amplicon and the nanoparticle is further isolated using a simple magnet.



Fig: Process flow of modified RT-LAMP assay and depiction of engineered nanoparticles.

### **ADVANTAGES:**

- 1. Highly sensitive: RT-LAMP assay can detect 10 copies of viral genomes.
- 2. Quick removal of contaminants (in 10 mins and by other methods takes 1 hour)
- 3. Single-step detection of viruses with reduced false positives results.
- 4. The method can be employed in point-of-care devices without high operational requirements.

### **APPLICATION:**

- 1. Detection and removal of carry-over contaminants of SARs-COV-2 viral genome.
- 2. The proposed method can also be extended to other pathogens and viruses.

**SCALE OF DEVELOPMENT:** Nanoparticles developed and tested at lab scale.

## **TECHNOLOGY READINESS LEVEL:** TRL 4

**IP STATUS:** Indian Patent Application (202111011611)