TECHNOLOGY DETAILS

i. About the Technology/Product/Process:

RT-LAMP is a relatively new amplification and detection protocol. Unlike RT-PCR which requires denaturation, annealing and extension at different temperatures the RT-LAMP reactions are incubated at a single temperature (isothermal reaction) usually at 65^{o}C for 30-40 minutes. Therefore, LAMP assays are easy to perform, rapid and may become cost-effective. In this invention, a novel colorimetric RT-LAMP assay has been developed for the detection of Nipah virus. The invention involves designing six novel sets of RT-LAMP primers, especially specific to the nucleocapsid (N) and matrix (M) gene of the Nipah virus. The conserved genomic segments amongst the isolates of Nipah virus from India and abroad by performing multiple sequence alignment. The RT-LAMP primers were designed on the basis of four key factors such as the melting temperature (Tm), stability at the 3' and 5' end of each primer (Delta G), GC content and ability to form secondary structures. The primers were synthesized and dissolved in specific proportions which is to be used in the RT-LAMP reaction. RT-LAMP results are read by naked eye as change of the color (pink to yellow) of the reaction mixture at the end of the incubation without the aid of any equipment. Unique points about the assay:

- One temperature amplification reaction, hence does not require a thermal cycler as in case of PCR or real-time PCR.
- The assay specifically detects two genes of Nipah virus (N and M gene).
- The results are interpreted visually by observing the color change. Sophisticated reading instrument not needed.
- As sensitive as the currently available commercial real time RT-PCR assays approved by ICMR.

ii. Need and utility of the Technology from Public health perspective:

Nipah virus (Nipah Virus, NiV) is a new paramyxovirus in recent years, can cause acute central nervous system disease in humans and animals. The Nipah virus infection is a Zoonotic disease transmitted to humans via Bats. The virus can also be transmitted through contaminated food or direct contact with infected individuals. There is no vaccine or other medical antiviral treatment available and the mortality rate is around 60 to 90%. Therefore, Nipah virus is classified under Biosafety Level 4 (BSL4) virus.

The development of a rapid molecular is of prime importance here because there are no effective field tests available currently for the detection of Nipah virus. This invention discloses a reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the detection of Nipah virus. The laboratory diagnosis of Nipah virus infection includes the following: virus isolation and identification, serum neutralization test (Serum neutralization test, SNT), enzyme-linked immunosorbent assay (Enzyme-linked immunosorbent assays, ELISA), RT-PCR detection, etc. All these detection techniques even though well-established they requires expertise and can be performed only in high-end laboratories. Therefore, the currently available diagnostic tests cannot be applied for surveillance and detection at the point of care. The present invention can be applied to the suspected clinical samples that can be used in the field as a rapid detection assay. This invention is easy to perform, rapid and the results can be interpreted visually and does not require any instruments.

iii. **Technology Readiness level (TRL)**

The NIV Mumbai Unit designed the LAMP Asssay. In the developer's lab, testing was completed, as the initial pilot study. In BSL-4, NIV, Pune (which is the only laboratory doing *Nipah virus* detection.), the assay's performance was compared to real-time PCR, yielding very positive outcomes. Real-time PCR assays approved by DCGI were used for the comparison. It was discovered that the developed LAMP assay for *Nipah virus* detection was just as sensitive and selective as the gold standard Real Time-PCR assay.

TRL 04/05

iv. **Validation Status and outcome:**

As a part of independent validation, 150 samples (Nipah Isolate, Spiked Human Nasal Swab/Throat Swab samples) were used in the BSL-4, NIV, and Pune (which is the only laboratory doing *Nipah virus* detection.) for performance evaluation of the RT-LAMP. Comparing the RT-LAMP assay to the Real Time PCR, the overall diagnostic sensitivity and specificity was 100% and 100%, respectively.

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Validation Report

Name of the Assay: RT-LAMP Assay for the detection of Nipah virus.
Application of the assay: Nipah virus molecular diagnosis.
Details of assay components: Primers (M gene & N gene), Warm Start Colorimetric LAMP Master Mix with UDG, Enhancer, Nuclease free water (NFW)

Objective: To determine the sensitivity and specificity of RT-LAMP Assay developed for detecting Nipah virus in comparison with TaqMan based Real time PCR assay.

Result of the panel tested

ICMR NIV, Pune	Nipah RT-LAMP assay		
Test	**Positive**	**Negative**	**Total number of samples tested**
Nipah Positive	75	0	75
Nipah Negative	0	75	75
Total	75	75	150

Sensitivity = 100%
Specificity = 100%
Comments on performance of the assay: Satisfactory
Conclusion: Satisfactory
(*Sensitivity and Specificity have been assessed in controlled lab settings*)
Remarks:

- The bench marking criteria of validation was as below:
  Sensitivity: ≥95%
  Specificity: ≥ 95%

Assay performance was satisfactory. Kit performance was found to be satisfactory using the validated panel of Nipah virus positive, negative samples.

This report is exclusively for Nipah virus RT-LAMP assay provided by ICMR NIV Mumbai unit.

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IP Filing Status/Publications

Patent: Development of a colorimetric isothermal (RT-LAMP) assay for rapid detection of *Nipah virus*

Indian Patent ApplicationNo: 202211057074, Dated 04/10/2023.

Inventors: Shyam Sundar Nandi (PI), Sonali Sawant, Upendra Lambe, Yadav Pragya, Shete-Aich Anita, Jagadish Deshpande.

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