A colorimetric isothermal (LAMP) assay for rapid detection of Monkeypox virus

TECHNOLOGY DETAILS

i. **About the Technology/Product/Process:**

The diagnosis of the Mpox is based on detection of an *Orthopoxvirus* genus specific gene and confirmation based on *Monkeypox virus* specific gene. The oligonucleotide primers have been designed targeting the genus specific B6R (Envelope protein) and species specific F3L (F3 protein which an enzyme double-strand RNA-binding domain) genes for the development of this assay. LAMP amplification reactions were incubated. The results were interpreted in the form of color change form pink to yellow only in 40 minutes time. The LAMP assay for B6R and F3L genes along with internal control Beta-actin gene were carried out in separate tubes. Unlike real-time PCR, this isothermal amplification technique does not require a thermal cycler. It is possible to conduct the experiment using a single temperature heating apparatus (65 ± 1^{o}C). The assay only takes forty minutes to complete. No further complex tools are needed to interpret the results; they may be comprehended visually. This technology is affordable. Field testing outside of the diagnostic laboratory may be made possible by this assay.

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

Monkeypox virus (Mpox) causes a smallpox-like disease in non-human primates and humans. This infection is endemic to central and western Africa. Mpox is divided into two genetically different groups, Congo Basin and West African Mpox, with the former being the more virulent. The invention discloses a loop-mediated isothermal amplification (LAMP) for rapid and low cost detection of *Monkeypox virus*. The principle of this assay is by using pH sensitive dyes to exploit the change in the pH resulting from proton accumulation while incorporation of dNTPs. Change in the color of reaction can be detected by naked eyes. No sophisticated instruments are required for the performance and results interpretation. By application of the LAMP for detection as described in this invention, the demerits of real-time PCR such as long detection period, complex operation, and sophisticated instrument requirement have been overcome. This assay is suitable to be performed as a point of care diagnostic assay at the health care centres, surveillance laboratories and other areas and has important significance for preventing and controlling the spread of *Monkeypox virus*.

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

The NIV Mumbai Unit designed the LAMPAsssay. In the developer's lab, testing was completed, as the initial pilot study. In BSL-4, NIV, Pune (which is the only laboratory doing *Monkeypox 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 M*onkeypox virus* detection was just as sensitive and selective as the gold standard Real Time-PCR assay.

iv. **Validation Status and outcome:**

As a part of independent validation, 160 clinical samples (NPS OPS Swabs, Lesion fluids) were used in the BSL-4, NIV, Pune (which is the only laboratory doing *Monkeypox virus* detection.) for performance evaluation of the LAMP. Comparing the LAMP assay to the Real Time PCR, the overall diagnostic sensitivity and specificity was 100% and 100%, respectively.

v. **IP Filing Status/Publications**

Indian Patent ApplicationNo:202211057074 entitled "Development of a colorimetric isothermal assay for detection of *Monkeypoxvirus*" has been filed on 04/10/2023.

Inventors: ShyamSundar Nandi (PI),SonaliSawant, UpendraLambe, Yadav Pragya, Shete-AichAnita,JagadishDeshpande.

****