

~~One eighty suspected infected serum samples collected from the local hospital were detected using this~~ Our assay ~~after nucleic acid extraction~~ was used after nucleic acid extraction to detect 180 suspected HIV-infected serum samples collected from the local hospital. All the serum samples were collected from ~~these~~ the patients ~~from~~ between March 2018 ~~to~~ and March 2019 and stored at ~~-~~ -90°C ~~after institutional ethics clearance.~~ This study was approved by the Institutional Review Boards of the Affiliated People's Hospital, Nourthern Medical University, Mandya. ~~Besides,~~ Then, comparison with conventional the PCR method was ~~carried out~~ performed, and all ~~these~~ samples were subjected to the real-time PCR assay ~~as previously described~~ [21].

Liver samples were collected ~~directly~~ immediately following euthanasia, ~~Followed by~~ and protein extraction was performed using lysis buffer supplemented with phosphatase and protease inhibitors (Thermo Scientific, USA). Protein concentrations were determined using the bicinchoninic acid ~~BCA~~ pProtein ~~a~~Assay kKit (Thermo Scientific, USA). Samples were denatured at 100°C for 10 ~~minutes~~ minutes after ~~mix~~ ing with sodium dodecyl sulfate ~~SDS~~ loading buffer. ~~Afterward~~ Then, the samples were loaded onto ~~into~~ sodium dodecyl sulfate ~~polyacrylamide gel electrophoresis~~ SDS-PAGE ~~gel~~ and transferred onto a nitrocellulose membrane. Blots were incubated at room temperature with 5% bovine serum albumin ~~BSA~~ in the Tris-buffered saline buffer for 1 ~~hour~~. The blots were then incubated overnight ~~in~~ at 4°C ~~with a~~ 1:2000 diluted rabbit anti-LDH antibody (ab52488, Abcam, UK) and ~~a~~ 1:2000 diluted mouse anti-β-actin antibody (A5441, Sigma, Germany) ~~at 4°C overnight.~~

~~Four μm-thick s~~ Sections (4-μm thick) were ~~incised~~ cut and rehydrated for immunohistochemical analysis ~~stry~~ using the standard avidin-biotin-peroxidase complex method ~~as previously described~~ earlier [17]. The 1:500 diluted rabbit anti-A~~gxt~~ GXT antibody (NBP1-89200, Novusbio, USA) was used as the

primary antiserum. All the sections were processed within a single~~one~~ assay and ~~underwent~~analyzed using the same procedure.