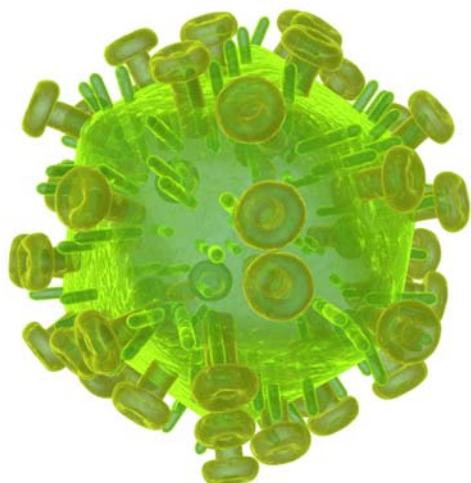




Multiplex Reverse Transcription-PCR for Detection of Viral RNA



DESCRIPTION

Early detection of viral infection improves treatment outlook and prevents further transmission of viruses such as HIV or human cytomegalovirus; however during the early stages of infection, patients may not yet express anti-viral antibodies or observable symptoms. This necessitates a test for low quantities of viral transcripts as an early indicator of infection. Tests to quantify viral load can also be used to evaluate the severity of a viral infection or as a screening test to verify safety of blood transfusion products

City of Hope has developed a method with a demonstrated ability to identify as few as 100 individual molecules of RNA from as little as 10 nanograms of cellular RNA. Reverse transcription PCR converts a target sequence of viral RNA into DNA, which then acts as a template for amplification by PCR. Simultaneously, a known quantity of synthetic reference RNA is included in the amplification process, so that after amplification the quantity of the target viral RNA can be determined by comparing relative signal strengths from a labeled hybridization probe(s). Furthermore, a unique construction of the reference RNA sequence imparts on it similarities to the target RNA. This creates an ideal reference reaction for accurate quantification while still creating amplification products that are distinguishable from each other.

The technology is compatible with a variety of probes, for example, those using radiolabeling, fluorophore or biotinylation markers, allowing the method to be applicable to a variety of assays ranging from southern blots to high throughput qPCR.

KEY ASPECTS

- Method for quantitative detection of target viral RNA sequences such as HIV or human cytomegalovirus (HCMV) by simultaneously amplifying reference RNA of a known quantity
- Reference RNA can be designed to share similar primary and secondary structure with the target RNA, improving accuracy and/or decrease complexity of the test assay
- Compatible with a variety of hybridization probes (e.g. radiolabels, fluorophores, and biotinylation)

INTELLECTUAL PROPERTY

Title	US Patent Number	Issued
Method for Amplification and Detection of RNA Sequences	8,227,191	7/24/2012

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