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Widely used virus assay shown unreliable when compared to other methods

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In the course of doing research on the mosquito-borne pathogens chikungunya virus (CHIKV) and o' nyong-nyong virus (ONNV), Virginia Tech researchers have discovered an inconvenient truth about an assay, strand-specific quantitative real-time PCR (ssqPCR), increasingly being used to detect and measure replicating viral RNA in infected cells and tissues. The method most labs are using for ssqPCR is unreliable.

The research appears in the Wednesday, October 14, 2009, issue of PLoS ONE, in the article, "Accurate Strand-Specific Quantification of Viral RNA," by Nicole E. Plaskon of Richmond Va., a Master of Science in life sciences candidate in the College of Agriculture and Life Sciences, and entomology Assistant Professors Zach N. Adelman and Kevin M. Myles, all with the Fralin Life Science Institute..

CHIKV has sickened millions of people in India and Africa in the last five years - 1.3 million in India alone. ONNV has also previously caused large outbreaks of human disease with cases numbering in the millions. In studying virus infection of the mosquito, the Virginia Tech researchers developed a novel assay that detects and measures anti-genomic copies of the viral genome. This differs from traditional assays that simply measure viral nucleic acids associated with infection, regardless of origin.

"The application of real-time PCR to the detection and quantification of specific strands of viral RNA is becoming an increasingly important tool

in the study of RNA viruses. As a result, multiple types of ssqPCR assays have been described and are in widespread use. However, no study has yet determined if the accuracy with which the different types of assays detect and quantify specific strands of viral RNA are equivalent. It turns out they are not, and the most frequently used method is the most error prone," said Myles.

"A less frequently used ssqPCR assay turned out to be more accurate," said Adelman.

"The fact that many labs have been using assays prone to error may have led to some wrong conclusions," Adelman said. "Using the more accurate assays will lead to more accurate conclusions and better science."

Although Myles and Adelman developed their assays for CHIK and ONNV, the results should help improve the design of ssqPCR assays for the study of other RNA viruses as well.

More information: The *PLOS One* (Public Library of Science) article is available on line at dx.plos.org/10.1371/journal.pone.0007468

Source: Virginia Tech (news : web)

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