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Critical components of diagnostic real time polymerase chain reaction: From sample preparation to extraction to amplification

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The real time polymerase chain reaction (qPCR) has now been widely used as one of the mainstream diagnostic assays for rapid detection of infectious diseases in animals due to its sensitivity and specificity. However, the interpretation of qPCR results can be challenging and must be interpreted in conjunction with the history, clinical signs and the evidence of disease. Performing qPCR assays involves multiple steps including sample preparation, nucleic acid extraction and amplification. This study aims at critical evaluation of each step based on our experience working with multiple animal disease viruses including avian influenza virus (AIV), classical swine virus (CSFV) and *Capripoxvirus* (CaPV). Diagnostic specimens were collected from experimentally and naturally infected animals including swabs (multiple types), blood and tissues (multiple types). Critical factors that influence the performance of qPCR include: Inefficient release of the virus from specimens (sample preparation), co-purification of naturally occurring PCR inhibitors (extraction) and PCR failure (amplification). The foremost challenge has been the false negative results due to PCR inhibition. Indigenous (beta-actin) or exogenously added controls (reverse transcribed RNA) were used to detect PCR inhibitors. The highest level of PCR inhibitors were detected in fecal samples (AIV-infected specimens) followed by blood, tissues and swabs (AIV and CSFV-infected specimens). Commercial extraction kits failed to completely remove PCR inhibitors from the clinical specimens. Commercial protocols were modified by adding an extra high-salt (NaCl-EDTA) washing step and the modified protocols were found to efficiently remove PCR inhibitors from the clinical specimens and subsequently improved the diagnostic sensitivity (DSe) of the qPCR assays. The choice of PCR kit (DNA polymerase) also had a large impact on the DSe (CaPVqPCR) due to the differences in their tolerance against the PCR inhibitors.

Biography

Amaresh Das has completed his PhD from Calcutta University, India and Postdoctoral Research from the Department of Biochemistry and Molecular Biology, University of Georgia. Currently, he works as a Microbiologist in the Reagents and Vaccines Services Section, Foreign Animal Disease Diagnostic Laboratory, NVSL, STAS, VS, APHIS, USDA, Plum Island Animal Disease Center, Orient Point, New York. He has published more than 20 scientific papers in reputed peer reviewed journals and wrote several chapters in books and proceedings. He also serves as a Reviewer for many reputed peer reviewed journals.

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