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Paper Poster Session
Respiratory virus diagnostics

Comparison of Copan MSwab™ rapid direct nucleic acid extraction to traditional nucleic acid extractions for the detection of enteric pathogens in stool samples with the R-Biopharm RIDA®GENE real-time PCR

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Background: The Copan MSwab™ is a molecular medium that has a formulation compatible with PCR master mixes and supports both viruses and aerobic bacteria cultures.
MSwab™ can be used with both rapid direct and traditional nucleic acid extraction methods for the detection of viruses and bacteria with molecular amplification assays. It is available in kits format, a medium tube associated with a flocked swab for specific collection sites. Clinical specimens collected in MSwab™ can be tested for the detection of viruses or bacteria by molecular assay and confirmed by culture. The objective of this study was to compare both rapid direct and traditional nucleic acid extractions methods for the detection of enteric pathogens in clinical stool samples using the RIDA®GENE real-time PCR assays

Material/methods: : In this study 150 known positive and negative stool samples, including adenovirus, rotavirus, norovirus, and *C. difficile,* were transferred into MSwab™ medium using a regular FLOQswabs™, and further diluted 1:10 in another MSwab™ tube. Aliquots of MSwab™ stool samples were used for traditional nucleic acids extraction with the PrepSEQ extraction kit on the AutoMate Express extractor (Life Technologies). Another 200ul aliquot of each MSwab™ stool sample was transferred into a microtube containing 150 µg of glass beads, vortexed, heated at 98°-100°C, and centrifuged at high speed for 2 minutes). Five microliters of extracted nucleic acids with both traditional and direct rapid methods were added to 20 ul of Master Mix and tested by real time PCR with RIDA®GENE kits for Clostridium difficile, Norovirus I & II and Viral Stool Panel II (R-Biopharm, Darmstadt, Germany) on the ABI 7500 Real Time PCR System.

Results: All *C.difficile* positive samples were detected in MSwab™ with 100% concordance between the standard extraction method and the rapid direct extraction method with the RIDA®GENE Clostridium difficile PCR assay. Good results correlation with the Adenovirus, rotavirus and norovirus positive stool samples was obtained with both direct rapid and traditional extraction methods using the samples further diluted 1:10 in MSwab with the RIDA®GENE Norovirus I & II and Viral Stool Panel II PCR assays. Diluting the stool samples with the direct rapid extraction method eliminated inhibition or interference of amplification due to the excess materials present in stool samples. No false positive or inhibition was detected with the negative stool sample with all testing methods.

Conclusions: The data demonstrated that the MSwab™ rapid direct nucleic acid extraction method with glass beads can be used for the detection of gastro enteric pathogens in clinical stool sample using RIDA®GENE Real Time PCR kits. The Copan MSwab™ direct rapid nucleic acid extraction method improves the turnaround time and is cost saving, compared to commercial extraction methods.