APPLICATION NOTE

Automation of Norgen's Pathogen /Viral Nucleic Acid Isolation Kit to Detect Viruses and Pathogens in Clinical Samples and a Variety of Bodily Fluids and Transport Media

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INTRODUCTION

Bacterial and viral pathogens have plagued human populations for much of history. Some posit that infectious diseases became more deadly as human inventions allowed for population crowding [1]. We are now trying to combat this with other human inventions such as vaccines, antibiotics, and anti-virals.

Detection and identification of these pathogens are important for surveillance, public health planning, and for treatment. Currently, molecular detection techniques are on the rise due to their ability to provide accurate results with significantly less time and effort compared to traditional culture-based methods. The most common nucleic-acid based tests are real-time quantitative PCR and next generation sequencing.

The COVID-19 pandemic further accelerated research in the area of molecular detection of pathogens and viruses. There have been developments and improvements in high throughput screening[2], as well as mobile [3] and at home molecular screening. Moreover, novel NGS sequencing techniques are being developed for new strain detection.

This paper describes the use of Norgen's new magnetic bead-based Pathogen/Viral Nucleic Acid Isolation Kit followed by quantitative-PCR to detect viral and bacterial pathogens in both laboratory and clinical samples. We demonstrate nucleic acid extraction and detection from a variety of bodily fluids including: saliva, urine, blood, and plasma, as well as a variety of swab preservation and transport media. We compared the Norgen kit to two different competitor's kits and demonstrated equal or better detection as measured by quantitative-PCR. Results from the clinical samples were significantly better, on average 6 Ct's lower, when the Nogen kit was used.

Materials and Methods

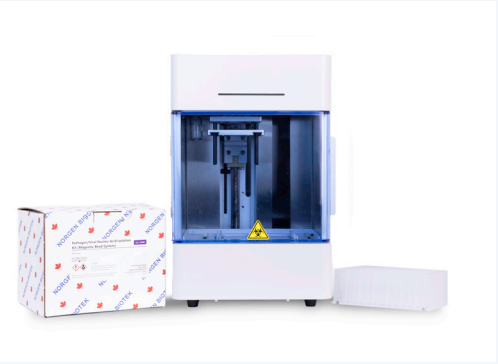
LABORATORY SAMPLES

In order to test the ability of the kit to extract total nucleic acids from a variety of sample types, we tested sample types and transport media that are most commonly used for virus and pathogen detection. For bodily fluids we tested: blood, plasma, saliva and urine. For transport media we tested, PBS, Viral Transport Medium, Norgen's Total Nucleic Acid Preservative (Cat. 69200, or Dx69200), and Zymo's DNA/RNA Shield. We took 200uL of each sample type and spiked them with $0.1x10^6$ HeLa cells, 10^6 *E. coli*, and $4x10^3$ COVID particles. For additional tests for gram positive pathogens we spiked each sample with 10^6 *S. aureus*.

Norgen's Total Nucleic Acid Preservation Tubes (Cat. 69200)

CLINICAL SAMPLES

Clinical samples were taken at a hospital in Southern Ontario in January 2024. Nasopharyngeal swabs were taken and immediately stored in a transport medium. Two of the swabs were stored in McMaster Molecular Medium (MMM) and the other six samples were stored in Copan UTM.



NUCLEIC ACID EXTRACTION

All laboratory samples were extracted using Norgen's Pathogen/Viral Nucleic Acid Isolation Kit (Cat. 72900), as well as a competitor's kit for comparison. In addition the clinical samples were extracted using a different competitor's kit. Extraction of the laboratory samples was automated on the IsoPure 96 magnetic rod automation system (Cat. 72705), and extraction of the clinical samples was performed on the IsoPure 16 automation system (Cat. 72715).

Quantitative PCR

LABORATORY SAMPLES

For the detection of COVID we used Norgen's reagents based on the CDCs protocols. Primers & probes for N1/N2 (Cat. TM 67101) with the corresponding positive controls (Cat. PC67102) and RT-Master Mix (Cat. 28113). For the detection of *Staphylococcus aureus* we used TaqMan PCR Detection Kit (Cat. TM29350). All PCR reactions were set-up as specified in the protocol and run on a QuantStudio 7Pro qPCR machine.

CLINICAL SAMPLES

For the clinical samples a multiplex qPCR system was used that tests for 10 targets and an internal control. The 10 targets include strains of Adenovirus, Influenza A/B, MPV, Covid, Parainfluenza, and RSV. The reactions were run on a Rotorgene Q machine.

Experimental workflow

Sample Preparation

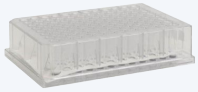
Clinicla Samples

A. Swab

Labratory Samples

A. Body Fluids

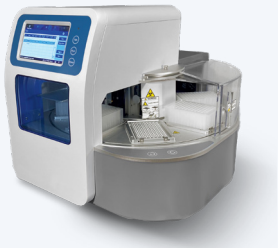
B. Transport Media



Prepare plates using Norgen & competitor kit regents.

Prepare processing plate for IsoPure96

Automated Total Nucleic Acid Extraction



Automated total Nucleic Acid extraction on IsoPure96.

Real time PCR on Extracted Nucleic Acid

Quantitative PCR and Data analysis.

Results

VIRAL PATHOGENS CAN BE DETECTED IN CLINICAL SAMPLES

Clinical Samples of Nasopharyngeal swabs were collected at a hospital and stored in either MMM or Copan UTM, nucleic acids were then extracted and analyzed for 10 viral targets. When using Norgen's Kit, viral pathogens were identified in all 8 samples. In one sample, Adenovirus and MPV were both identified when the Norgen kit was used, whereas the competitors kit did not detect the MPV in that sample. Moreover, the Ct values were on average 6 cycles lower when the Norgen kit was used for extraction, ranging from 2-10 cycles lower depending on the sample.

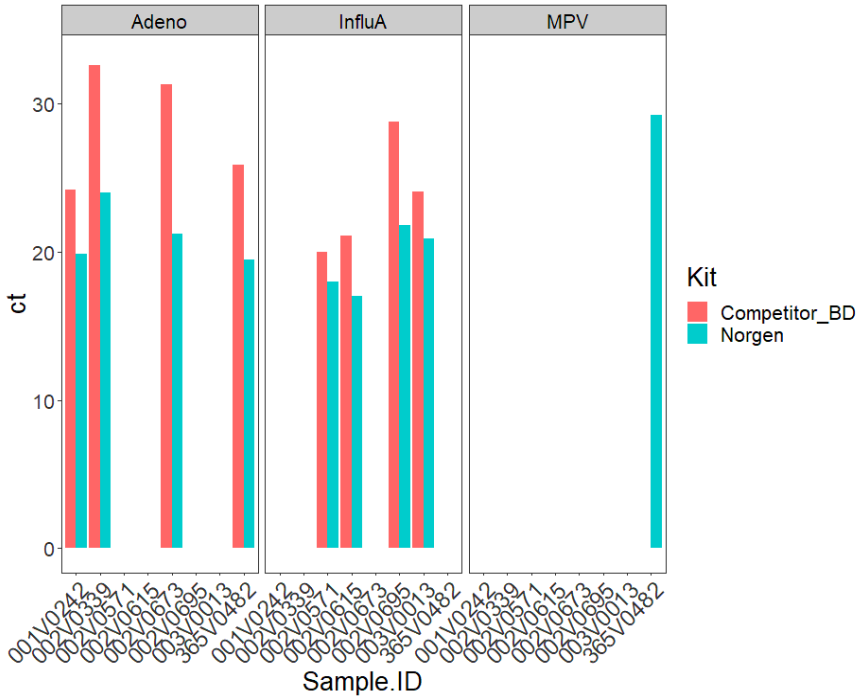


Figure 1 - Multiple viral pathogens were detected in clinical samples with Norgens Ct values being significantly lower than those when a competitors kit was used.

BOTH DNA AND RNA CAN BE EFFICIENTLY EXTRACTED FROM MANY BODILY FLUIDS AND TRANS-PORT MEDIA.

Quantitative PCR detection of DNA (GAPDH) and RNA (RNaseP) housekeeping genes shows that the Norgen kit can extract DNA and RNA from a variety of bodily fluids. Nucleic acids were extracted from blood, plasma, saliva and urine, and all resulted Cq values much below detection.

There were different yields based on the different sample types. These trends were the same for DNA and RNA and between the two different kit types. There were the highest yields, thus lowest Cq and detection limit when using Saliva samples, the next highest yield was from blood, then urine and there was the lowest yields from plasma samples. When comparing between the two different kits, the Norgen kit had a lower detection limit (lower Cq) than the competitor kit for most sample types, for saliva the detection limit was the same within deviation for both kits. (Figure 1).

A variety of different swab types can also be used to detect viruses and pathogens, for example, buccal, throat, nasal, skin, and vaginal. These swabs are preserved and transported in a variety of medias including: Norgens Total Nucleic Acid Preservative (Nor_TNA), Phosphate Buffered Saline (PBS), Viral Transport Medium (VTM) or Zymo's DNA/RNA shield (Shield). There was less variability in the yields between the different transport mediums. And comparisons between the kits show similar yields for most of the transport media. (Figure 2).

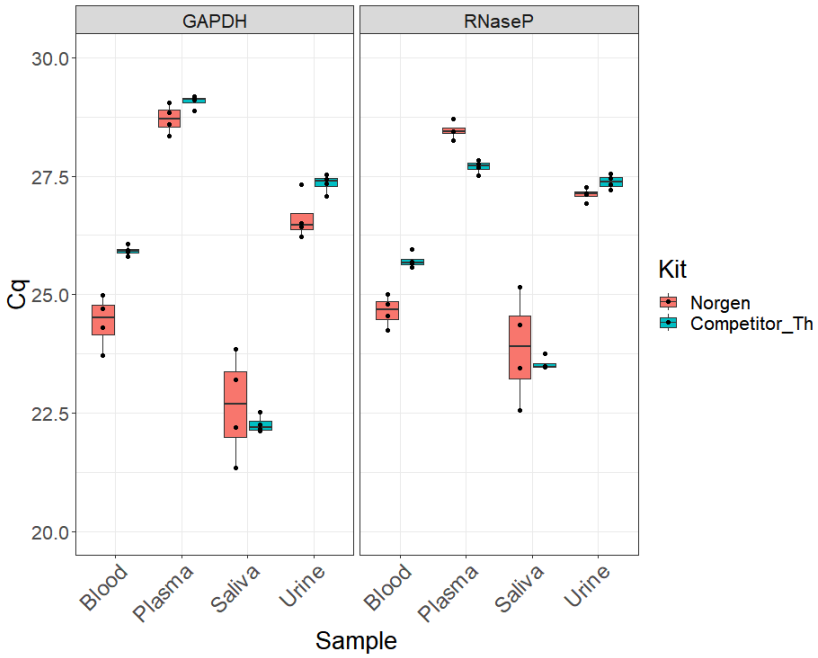


Figure 2 - Both DNA and RNA can be extracted from a variety of bodily fluids

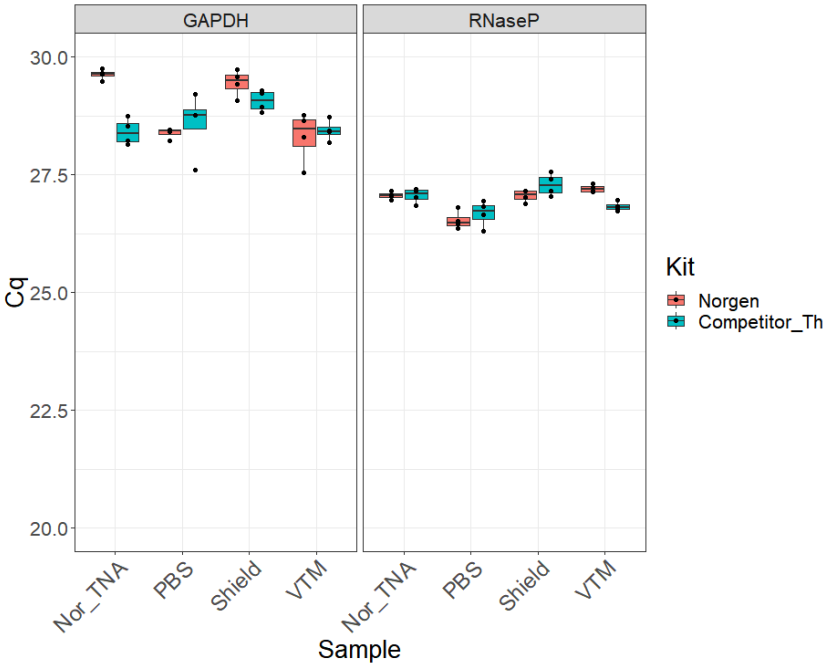


Figure 3 - Both DNA and RNA can be extracted from a variety of transport media

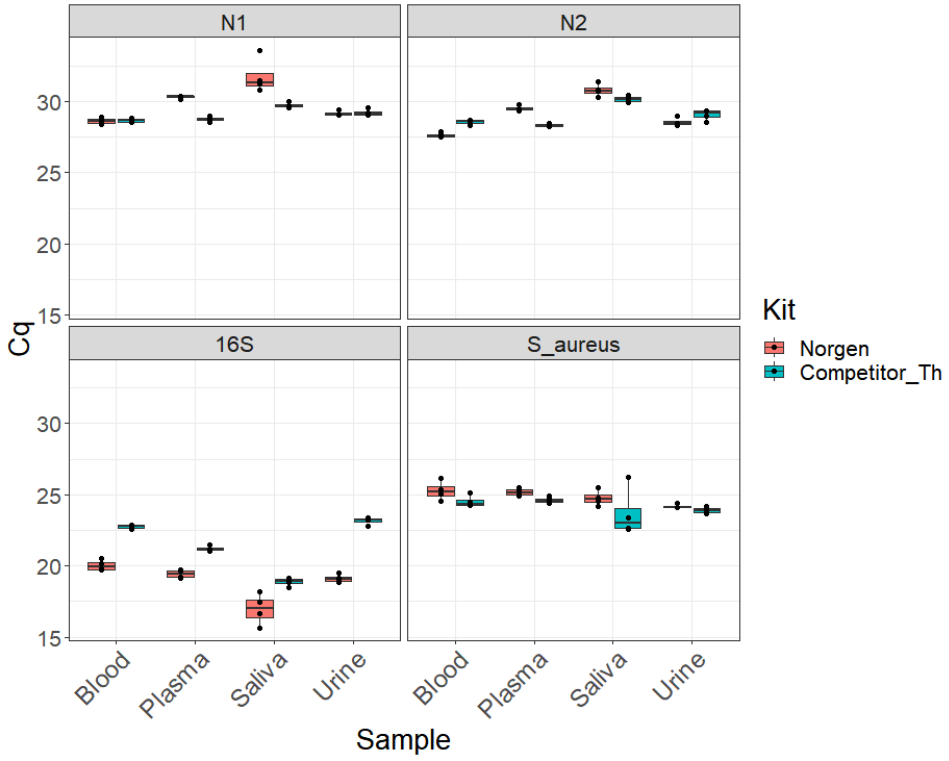


Figure 4 - Detection of Pathogens in Different Bodily Fluids

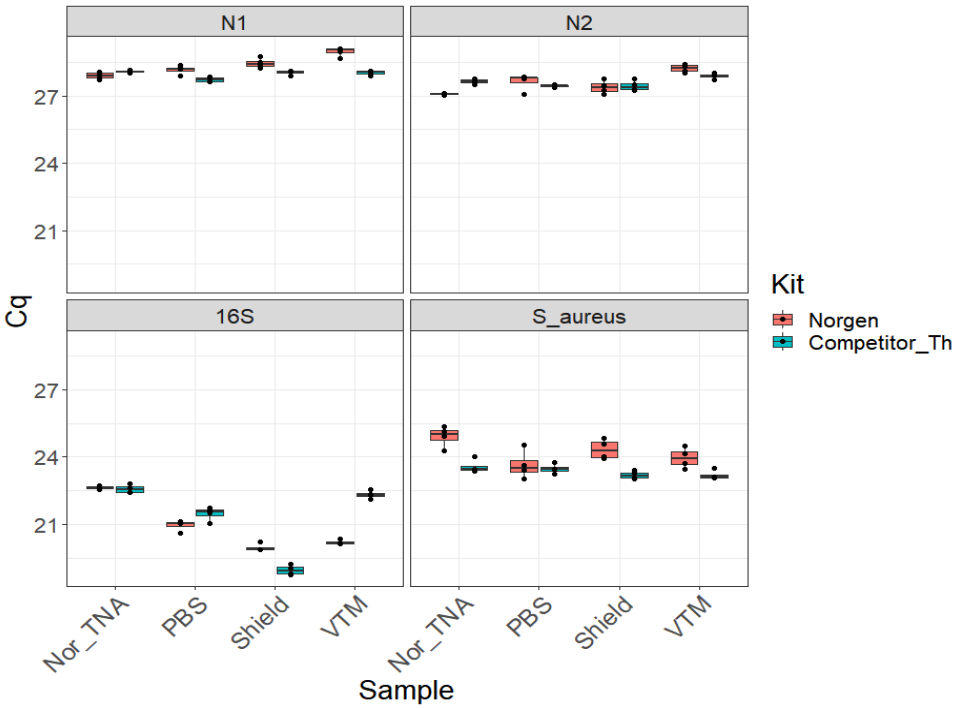


Figure 5 - Detection of Pathogen Nucleic Acids When extracted from Different Transport Media

NUCLEIC ACIDS EXTRACTED USING NORGENS KIT CAN BE USED FOR PATHOGEN DETECTION, WITH RESULTS SIMILAR TO A COMPETITOR

Next we compared the detection of COVID RNA from a variety of different bodily fluids. The detection of the N1 and N2 genes by qRT-PCR was similar between Norgen and the competitor's kit. In addition to detection of RNA viruses, we performed detection of DNA markers for bacterial pathogens. The 16S gene proves as a surrogate for general bacterial pathogens. In general, the detection of bacterial 16S was better with the Norgen kit as demonstrated by Cq. We also tested a modified version of the protocol with a lysozyme treatment to detect the gram positive bacterium *S. aureus*. The detection of *S. aureus* was similar across the different bodily fluids, as well as similar between extractions using the Norgen kit, and those using the competitor kit. (Figure 4.)

We next compared the detection of COVID RNA from a variety of different transport media. The results are similar to those with the bodily fluid samples. The detection of the N1 and N2 genes by qRT-PCR was similar between Norgen and the competitor's kit. In addition to detection of RNA viruses, we performed detection of DNA markers for bacterial pathogens. The 16S gene proves as a surrogate for general bacterial pathogens. The detection of bacterial 16S genes was similar between the two kits. We also tested a modified version of the protocol with a lysozyme treatment to detect the gram positive bacterium *S. aureus*. The detection of *S. aureus* was similar across the different bodily fluids as well as similar between extractions using the Norgen kit, and those using the competitor kit. (Figure 5.)

Conclusions and Summary

In conclusion, Norgen's new Pathogen/Viral Nucleic Acid Isolation Kit (Magbead Based System) can extract viral and bacterial DNA and RNA from a variety of bodily fluids including blood, plasma, saliva and urine. The kit is also compatible with a variety of transport media and thus can be used with swabs from a variety of preservation devices. The eluate that is obtained is of good quality and can be used for qPCR detection of the pathogens and viruses. The results obtained were also similar to when the same samples were processed using the kits of a leading competitor. Moreover, this kit can be easily automated. This paper shows automation on the Kingfisher-like IsoPure magnetic rod extraction system, however, the protocol can also be easily adjusted for use with liquid-handling automation systems such as Hamilton, Tecan, OT-2, or EppMotion.

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