ZYMO RESEARCH

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Automated Viral RNA Extractions from Plasma and Serum

ABSTRACT

Viral RNA extraction is ubiquitous in the diagnostic laboratories as it is the first step in diagnostic tests. The increasing popularity of viral DNA and RNA extractions has increased the need for an automated viral extraction solution to streamline and expedite workflows. To address this need, Zymo Research and Tecan have developed the DreamPrep NAP, a completely validated, walk away automated workflow for extracting viral DNA and RNA, even from low yielding sample types. This is demonstrated here by extracting varying abundances of HIV-1 that were spiked into serum and plasma samples. This was processed using the *Quick*-DNA/RNA™ Viral MagBead kit workflow scripted on the DreamPrep NAP. The automated extraction was able to detect as low as 60 viral copies and had a higher total RNA recovery when compared to manual extractions with no signs of cross contamination between samples. The DreamPrep NAP provides a complete solution to automated viral DNA and RNA extractions.

INTRODUCTION

Viral RNA extraction is becoming ubiquitous in diagnostic laboratories, as it is the first step in many viral diagnostic tests. The growing popularity of viral DNA and RNA extractions has increased demand for an automated viral extraction solution to streamline and accelerate workflows.

Sensitivity and consistency are two crucial requirements for any viral DNA/RNA purification system, due to very low amounts of the virus present in samples. Detecting viral RNA in such low concentrations requires a robust and reliable extraction system capable of binding very low amounts of nucleic acid and delivering pure, high quality nucleic acids for sensitive downstream applications.

The *Quick*-DNA/RNA™ Viral MagBead Kit was developed to provide a high throughput solution for extracting both DNA and RNA from viral samples. It specifically lyses viral particles, allowing purification of nucleic acids from as little as 60 viral copies.

In a collaborative effort between Tecan and Zymo Research, this technology has been automated on the DreamPrep NAP workstation to extract high quality viral DNA/RNA ready for all complex and sensitive downstream applications, including next generation sequencing, hybridization, and RT-qPCR (Figure 1).

MATERIALS AND METHODS

The *Quick*-DNA/RNA™ Viral MagBead extraction workflow was automated on the DreamPrep NAP workstation featuring Zymo Research, a system based on the Fluent® 480 Automation Workstation in combination with FluentControl™ GX Assurance Software. The system is configured for nucleic acid extraction workflows using magnetic bead-based procedures. An integrated

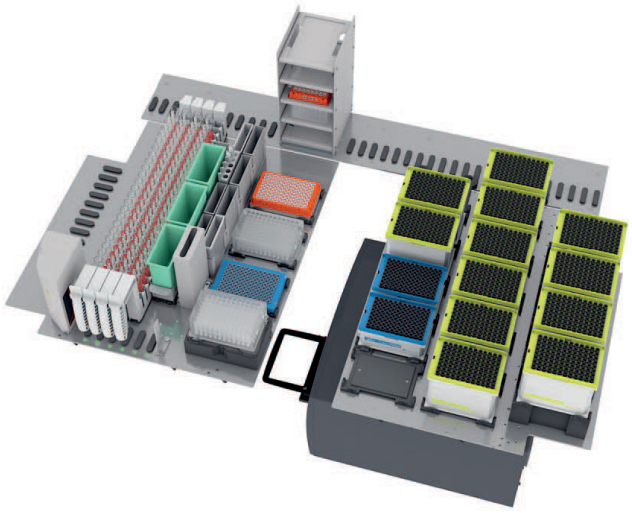

Figure 1: Configuration of the DreamPrep NAP featuring Zymo Research worktable.

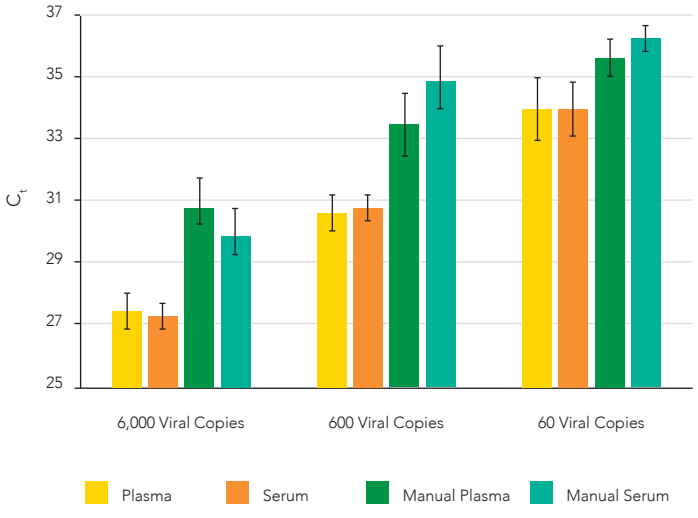

Figure 2: Manual Benchmarking. Viral RNA isolated from plasma and serum samples using the *Quick*-DNA/RNA™ Viral MagBead kit scripted on the DreamPrep NAP. Image shows average CT values (n = 4 +/- SD). Eluates from each well were amplified using RT-qPCR using GoTaq qPCR 2x Mix and GoScript RT Mix for 1-Step RT-qPCR (Promega).

Infinite® 200 PRO reader in M Nano+ configuration allows quantification and normalization following nucleic acid extraction. The system is equipped with an Air Flexible Channel Arm™ (Air FCA), a Robotic Gripper Arm™ (RGA), Fluent ID™ and a handheld barcode scanner (Honeywell) for sample and reagent identification, a BioShake™ D30-T elm (QInstruments) for heating and shaking, and a Magnum FLX® Enhanced Universal Magnet Plate (Alpaqua).

The automated workflow was tested using the following general protocol. First, standard HIV-1 controls were prepared by spiking in 10 µl (~6,000 viral copies) of AcroMetrix™ HIV-1 High Control (Thermo Fisher) into 190 µl of plasma or serum. These controls were serially diluted 1:10 (600 copies) and 1:100 (60 copies) to imitate samples of low copy virus.

200 µl of plasma or serum HIV-1 spike-in samples were then added to a 2 ml Nunc™ 96-Well DeepWell™ Plate (Cat. no. 278743) (n = 4) and processed using the *Quick*-DNA/RNA™ Viral MagBead Kit on the DreamPrep NAP. Viral RNA from each sample was eluted in 50 µl of DNase/RNase-Free Water. Eluates from each well were amplified using RT-qPCR using 2x GoTaq® qPCR Mix and GoScript™ RT Mix for 1-Step RT-qPCR (Promega).

RESULTS AND DATA ANALYSIS

Benchmarking of HIV-1 in plasma and serum

Cycle threshold (CT) values from the automated workflow were compared to manually extracted plasma and serum samples (Figure 2).

Evaluating extraction efficiency for low titer samples

CT values for each concentration of automatically extracted serum and plasma sample were plotted on a line graph, and the R2 values were used to check the efficiency and linearity of the process (Figure 3).

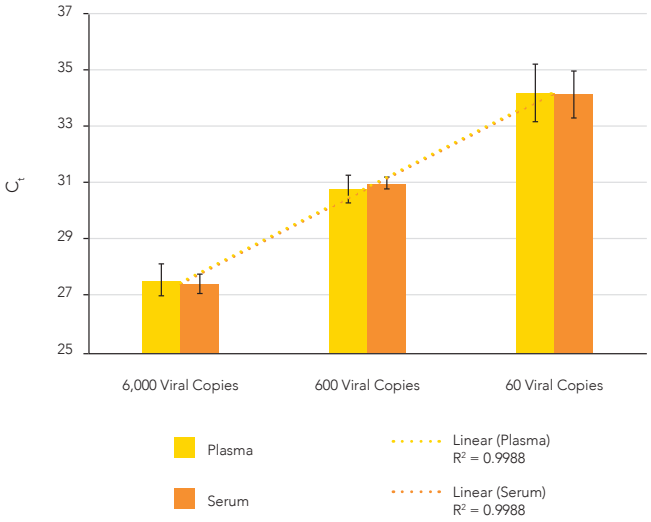

Figure 3: Linear Recovery of Low Titer Samples. Viral RNA isolated from plasma and serum samples containing 6,000, 600, and 60 viral copies. Viral RNA was extracted using the *Quick*-DNA/RNA™ Viral MagBead kit scripted on the DreamPrep NAP. Image shows average CT values from each sample (n = 4 +/- SD). Eluates from each well were amplified using RT-qPCR using GoTaq qPCR 2x Mix and GoScript RT Mix for 1-Step RT-qPCR (Promega) and compared against each group to check for linearity (R^2 = 0.99).

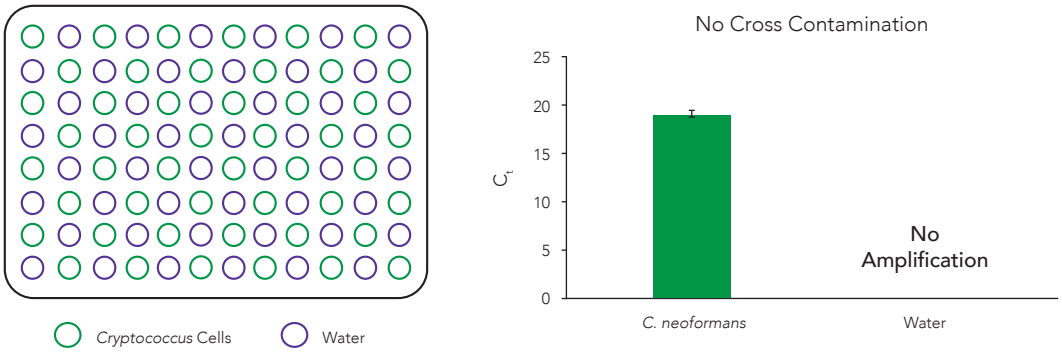

Figure 3: No Cross Contamination. *Cryptococcus neoformans* and DNase/RNase free water was added to every other well across a 96 well plate in a checkerboard pattern. All 96 wells were extracted using the *Quick*-DNA/RNA™ Viral MagBead system scripted on the DreamPrep NAP. Eluates from each well were amplified via qPCR using the Femto DNA Quantification kit. Wells containing both *C. neoformans* and water were analyzed to check for cross contamination of *C. neoformans* into the wells filled with water.

Checking for cross-contamination

Cross-contamination between each well was analyzed by adding Cryptococcus neoformans and nuclease-free water to every other well in a checkerboard pattern across a 96-well plate. Eluates from each well were amplified by qPCR using the Zymo Research Femto™ DNA Quantification Kit on the CFX96™ Real-Time System (BioRad Laboratories) to check for C. neoformans DNA in water-filled wells, demonstrating that no cross-contamination occurred (Figure 4).

SUMMARY

The DreamPrep NAP workstation featuring Zymo Research offers complete walkaway extraction of viral DNA and RNA from plasma or serum samples.

The results presented here demonstrate efficient recovery of viral DNA and RNA in a consistent and reproducible manner. The automated extraction workflow is equivalent to manual extraction in terms of total nucleic acid recovery, successfully extracting viral RNA from samples containing as little as 60 viral copies, with lower CT values. This workflow shows no cross-contamination and can achieve high quality viral DNA from a variety of samples (eg. plasma and serum). The resulting viral RNA can be used for sensitive downstream applications, such as RT/qPCR and next generation sequencing.

About the Authors

Shaun Veran is an Application Specialist at Zymo Research with a degree in Microbiology, Immunology, and Molecular Genetics from the University of California - Los Angeles. He specializes in high-throughput DNA automation, research and design, and product development.

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Acknowledgments

This protocol was developed by in collaboration with Tecan (www.tecan.com) and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application. For further information, visit www.zymoresearch.com.