APPLICATION NOTE

EFFICIENTLY AUTOMATING HIGH-THROUGHPUT VIRAL RNA PURIFICATION.

Automated Viral RNA Extraction Workflow for Omega Bio-tek's Mag-Bind® Viral RNA Xpress Kit on Tecan DreamPrep® NAP.

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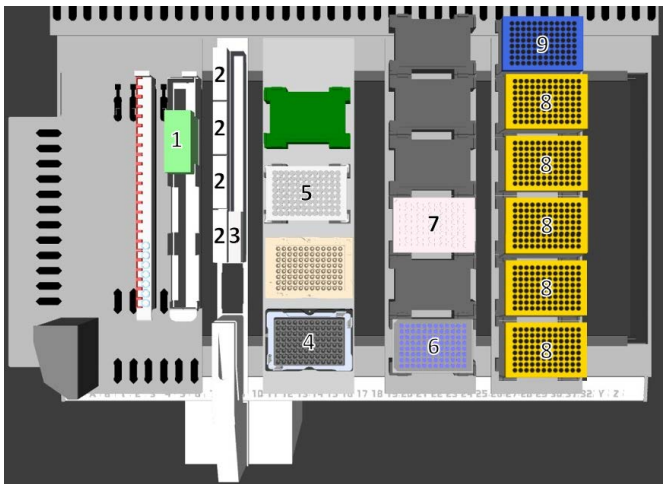
INTRODUCTION.

Viral nucleic acid extraction is a critical, first step in developing and realizing strategies for detection of both known and novel viral agents from biological specimens, environmental samples, and more. The demand for high-throughput, automated sample extraction workflows has become indispensable for achieving broader and more effective implementation. Omega Bio-tek's Mag-Bind Viral RNA Xpress Kit (M6219-2304) performs rapid and reliable isolation of viral RNA from nasopharyngeal (NP) swab specimens that are dry or in viral transport media (VTM). When automated on the Tecan DreamPrep NAP workstation, provides researchers and scientists with a high throughput, walk away viral extraction workflow with minimal manual intervention. In this application note, we present the automated workflow with performance data in terms of sensitivity of viral RNA detection. The assay was further assessed for potential instrument-based cross-contamination across different wells of the plate. Our results indicate that this workflow efficiently extracts viral RNA from ninety-six 200 µL samples arrayed in a 96-well format in under 100 minutes.

DreamPrep NAP worktable Layout.



1. 320 mL Trough for Liquid Waste
2. 100 mL Reagent Troughs
3. 25 mL Reagent Troughs
4. BioShake™ D30-T Elm
5. Alpaqua Magnum FLX® Magnet Plate Magnet
6. Nunc™ 96 2.0 mL Deep-well Sample Plate
7. Standard 96-well PCR Elution Plate
8. 1000 µL Filtered DiTi SBS format
9. 200 µL Filtered DiTi SBS format

Figure 1: DreamPrep NAP deck layout containing all the elements required for extraction of viral RNA from a spiked cell suspension.

MATERIALS AND METHODS.

The workflow to extract and purify viral RNA from 1-96 200 µL samples was automated using a DreamPrep NAP workstation based on a Tecan Fluent® 480 platform, equipped with an eight-channel Air Flexible Channel Arm™ (Air FCA), a Robotic Gripper Arm™ (RGA), a BioShake D30-T elm (QInstruments) for heating and shaking, and a Magnum FLX Enhanced Universal Magnet Plate (Alpaqua), as well as all the consumables required to process a batch of 96 samples (deck layout shown in Figure 1).

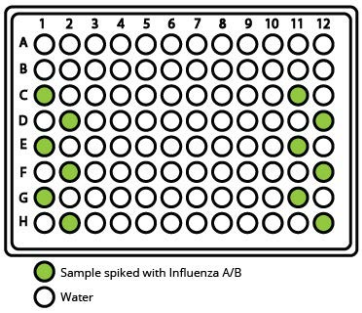
Mag-Bind Viral RNA Xpress Kit was used to purify high-quality viral RNA free of proteins, nucleases, and impurities. The Kit is magnetic bead-based offering reliability and scalability to the workflow. Viral RNA was extracted from twelve 200 µL samples containing 12,500 HEK293 cells spiked with 75 µL of ZeptoMetrix's NATtrol™ Influenza A/B Positive Control Standard. The samples were arrayed in a strategic pattern in a 96-well plate with alternate wells containing water to assess the cross-contamination as part of the extraction protocol as shown in Figure 2. The DreamPrep NAP was programmed to perform various liquid handling and magnetic bead-based tasks as required by the Mag-Bind Viral RNA Xpress Kit for the extraction of viral RNA. All consumables and carriers were placed onto the instrument deck configured as shown in Figure 1. The extraction workflow was fully automated starting with the sample aliquot in the 96-well plate to the final eluted product. Viral RNA was eluted in 60 µL volume and a real-time PCR was carried out using Influenza B-specific primers at 2 µL and 4 µL template amounts in a 20 µL reaction volume to derive information about sensitivity of detection. A qPCR plate with samples arrayed in the same format as the extraction plate was also subjected to real-time PCR using 2 µL template amount to assess cross-contamination.

RESULTS AND DISCUSSION.

The automated extraction with the DreamPrep NAP took approximately 100 min for processing 96 samples of 200 µL each. The results of the real-time PCR conducted using 2 µL and 4 µL of eluate as the template amounts are displayed in Figure 3. The Ct values at both template amounts indicates positive amplification and detection of Influenza virus even at template amount as low as 2 µL.

The layout for the cross-contamination study and subsequent results qPCR results in a 96-well plate format are as shown in Figure 2. Eluates in each well were amplified using qPCR specific for Influenza B virus to identify any well-to-well contamination across the 96-well plate during extraction process as well as qPCR setup. The Ct values exhibited in Figure 2 show that there is no cross contamination of purified RNA into wells containing water when using Omega Bio-tek's workflow developed for the Mag-Bind Viral RNA Xpress Kit on the DreamPrep NAP.

96-well plate map showing location of samples.



A	U	U	U	U	U	U	U	U	U	U	U	U
B	U	U	U	U	U	U	U	U	U	U	U	U
C	31.89	U	U	U	U	U	U	U	U	U	31.79	U
D	U	31.54	U	U	U	U	U	U	U	U	U	31.52
E	31.95	U	U	U	U	U	U	U	U	U	31.39	U
F	U	31.45	U	U	U	U	U	U	U	U	U	31.84
G	31.49	U	U	U	U	U	U	U	U	U	31.87	U
H	U	31.56	U	U	U	U	U	U	U	U	U	31.85

Figure 2: 96-well plate map showing the location of samples on the sample plate and subsequent qPCR amplification plate. The Ct values were only detectable in wells containing samples, illustrating no cross-contamination from Omega Bio-tek's automated protocol. (U: Undetermined).

Average Ct values indicate positive amplification of viral RNA at both template amounts.

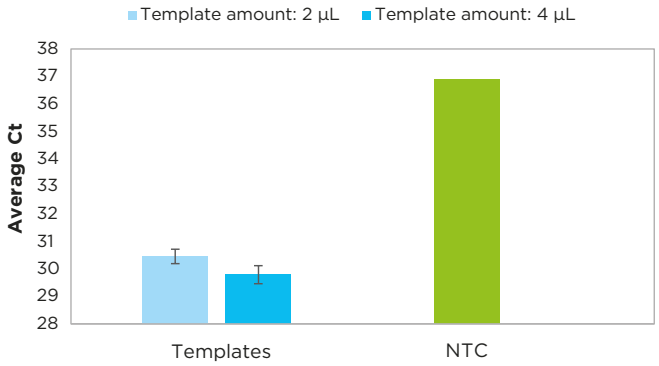


Figure 3: 200 µL sample containing 12,500 HEK293 cells spiked with 75 µL of ZeptoMetrix's NATtrol Influenza A/B Positive Control Standard was subjected to real-time PCR at template amounts, 2 µL and 4 µL and. The average Ct values post RT-qPCR indicate the positive amplification and detection of Influenza virus at both the template amounts tested compared to the No Template Control (NTC).

CONCLUSIONS.

Addressing the growing need for rapid and high-throughput viral RNA purifications, Omega Bio-tek's Mag-Bind Viral RNA Xpress Kit provides an efficient solution by optimizing the extraction workflow, using the Tecan DreamPrep NAP lab automation workstation. This streamlined method enables the processing of ninety-six 200 µL samples in 100 minutes, beginning with samples in a 96-well deep well plate.

The obtained results robustly support the kit's utility for viral detection through RT-PCR and next-generation sequencing, highlighting its effectiveness in meeting the demands of timely and efficient viral RNA purification.

ORDERING INFORMATION.

Product No.	Description
M6219-384	Mag-Bind® Viral RNA Xpress Kit (4 × 96 preps)
M6219-2304	Mag-Bind® Viral RNA Xpress Kit (24 × 96 preps)

ABOUT THE AUTHORS.

Kiranmai Durvasula *(PhD) is a Product Manager at Omega Bio-tek, Inc., leading the efforts to develop and deliver novel nucleic acid extraction products supporting the fields of basic and translation research, diagnostics, pharmacogenomics, etc. Kiranmai obtained her PhD in Chemical Engineering from University of Florida, Gainesville, and received postdoctoral training in cell-based therapies for treatment of insulin-dependent diabetes in Dr Athanassios Sambanis's laboratory at Georgia Institute of Technology. She has authored several peer-reviewed journal papers and is passionate about translating fundamental research platforms into practical technologies.*

Sara Amirahmadi *is a geneticist by training, she holds a BS in Genetics and an MS in Immunology & Bacteriology. Her skills in genetic analysis and laboratory methodologies were honed during her five-year tenure at Omega Bio-tek. Over the past three years, she has been working as a field application scientist, where her focus has been on automation and streamlining processes to achieve innovative outcomes. Beyond the lab, she finds balance in yoga and the world of books, nurturing both her body and mind.*

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