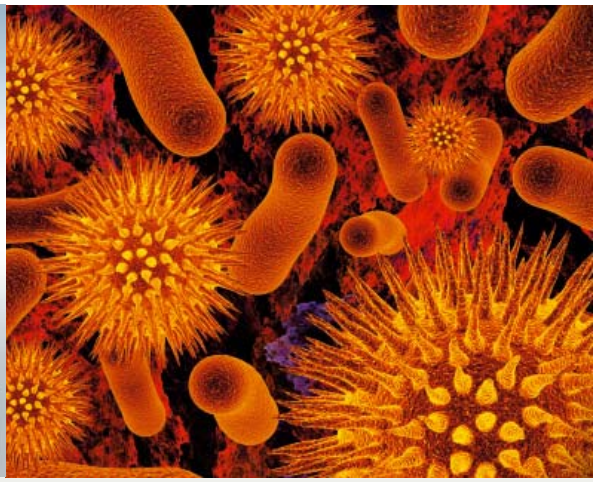

Viral nucleic acid extraction at high recovery yields

High yields and excellent purity viral nucleic acid extraction with the MACHEREY-NAGEL NucleoMag® 96 Virus kit on a Freedom EVO® platform.

Introduction

The isolation of viral nucleic acids (NA) plays a pivotal role in several life science areas, including epidemiology, immunology and virology. The genetic code of a virus needs to be known in order to develop effective treatments or vaccines. Important applications include monitoring the health of livestock and controlling food quality, especially during outbreaks of diseases such as Avian Influenza in birds or Bluetongue disease in sheep, goats or cattle, where it is vital to rapidly diagnose the correct subtype and to investigate the genetic variability of the viral nucleotide sequences.

However, the throughput, quality and quantity of viral NA preparations are often limiting factors for downstream analysis. In order to fulfil the need for efficient extraction of high quality DNA from serum or plasma samples, MACHEREY-NAGEL has developed the NucleoMag 96 Virus kit. The magnetic bead-based extraction process delivers NA of excellent purity and high yields, keeping the workflow very flexible with regard to sample numbers.

Tecan and MACHEREY-NAGEL have now combined efforts to provide a flexible automated solution for the reliable isolation of viral DNA and RNA, without compromising on yield or purity and with low running costs. The workflow is highly flexible and increases walkaway time. Common risks, such as contamination, carry-over and manual errors are reduced to a minimum, while the reproducibility of the procedure is maximized. Sample tracking improves sample security even more and consequently benefits the overall process security.

The excellent purity of the extracted NA is shown both in real-time PCR as well as in gel electrophoresis experiments. The results are highly reproducible with a CV of 1.42 %, demonstrating the robustness of the automated procedure.

Overall, full automation of the NA extraction procedure on a Tecan Freedom EVO workstation streamlines workflows and provides reproducible viral NA extraction of excellent purity.

Material and method

Equipment

The Freedom EVO liquid handling workstation can be equipped with a 2-, 4- or 8-channel liquid handling arm with disposable tip adaptors and a lower DiTi eject option to reduce cross-contamination. For medium throughputs of up to 48 samples per batch, a Te-MagS™ module is implemented (Fig. 1). High throughputs can be achieved using a magnetic separator, eg. MACHEREY-NAGEL's NucleoMag SEP positioned on a regular microplate carrier, a Te-Shake™ module for heating and mixing of the samples and a robotic manipulator arm (Fig. 2). Table 1 compares the different equipment requirements for the different throughput scenarios.

	Medium throughput	**High throughput**
Sample numbers	1 - 48 samples per batch	1- 96 samples, or multiples of 96 per batch
Batch time	2 h 20 min	3 h 45 min
Equipment Tecan	• Freedom EVO 100 platform, 8-channel liquid handling arm configured for disposable tips, 1,000 µl syringes, stainless steel deck and safety panel set • tube, trough and disposable tip carriers • washstation with waste • disposable tips (filtered) 1,000 µl, 200 µl and 100 ml troughs • Freedom EVOware® Standard software package	
	• Te-MagS	• robotic manipulator arm • Te-Shake • microplate carriers
Equipment MACHEREY-NAGEL	• NucleoMag 96 Virus kit	• NucleoMag 96 Virus kit • Square-well blocks • NucleoMag SEPMagnetic separator

Table 1 Overview of equipment for different throughput requirements

Automated workflow

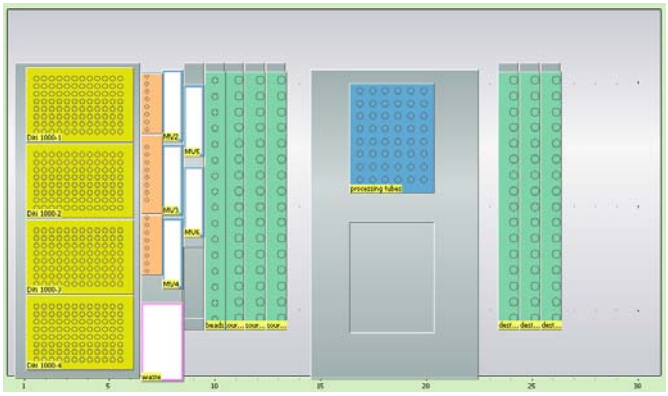

Figure 1 Medium throughput - Worktable layout with the Te-MagS module

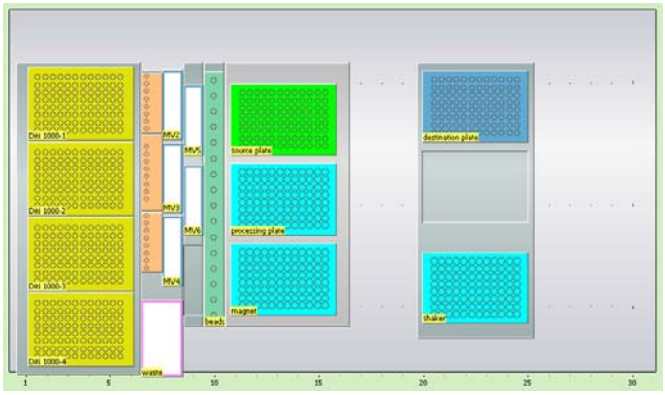

Figure 2 High throughput - Worktable layout with Te-Shake and magnetic separator from MACHEREY-NAGEL

The automated workflow includes a proteinase K digest and subsequent extraction of NA with magnetic beads directly from serum or plasma samples. In this application note, special emphasis is given to method optimization with the DNA virus phage T7 and the RNA virus phage MS2. The manual process was compared to the automated process in terms of yield and purity. The configuration and scripting of the Tecan workstation have been optimized to minimize the risk of cross-contamination and maximize the yield of nucleic acids.

Results

Automation of the NucleoMag 96 Virus kit on the Freedom EVO workstation allows fast and reliable purification (Fig. 3) of viral DNA and RNA. The yield and purity are equivalent to the manual method (Figs. 2 and 4), whereas the speed is greatly increased by the automated process. On average the extraction of viral NA from eight samples by a manual process takes a trained technician about an hour (beads resuspended by pipetting up and down). The automated process with the Te-MagS also takes an hour, but for 24 samples. The automated extraction of 96 samples takes 3 hours 45 minutes for viral DNA, including the proteinase K digest (with the Te-Shake and magnetic separator).

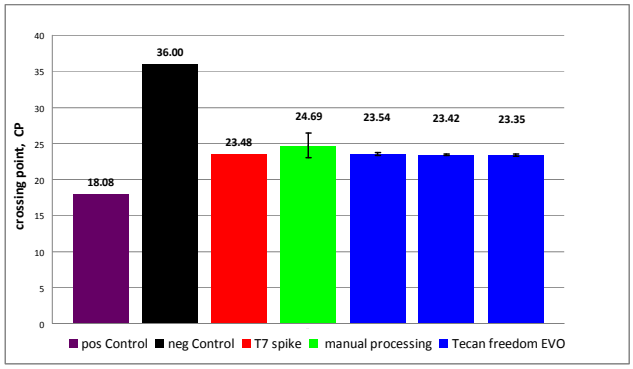

Figure 3 T7 DNA spike recovery of manual and automated methods

The yield of NA extraction was analyzed by measuring the crossing points of a T7 phage-specific PCR fragment by real-time PCR (Fig. 3). The input amount of viral NA shows a crossing point of 23.5 and the yield by manual NA extraction shows a crossing point of 24.7. The yield with the automated process is slightly higher, shown by a crossing point of around 23.4 even after fewer cycles.

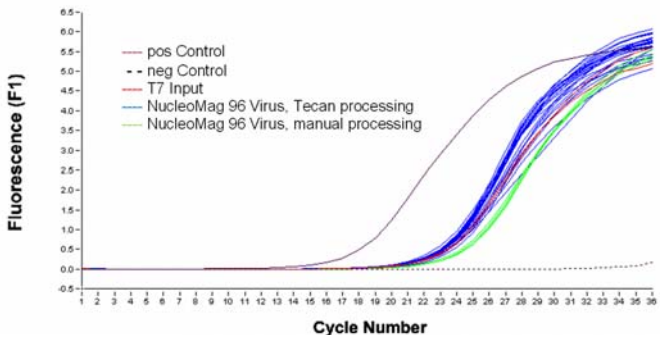

Figure 4 Amplification of a PCR fragment of DNA from the T7 phage by real time PCR, data generated with the magnetic separator from MACHEREY-NAGEL together with the robotic manipulator arm from Tecan

The diagram in Fig. 4 shows the crossing points and amplification curves of a PCR fragment, monitored by real-time PCR. The high consistency of the purification process is shown not only by the crossing points lying close to each other, but also by the high degree of similarly shaped amplification curves of all DNA purified by the automated process. Figure 3 underlines the highly reproducibility of the automated DNA extraction between 96 identical samples.

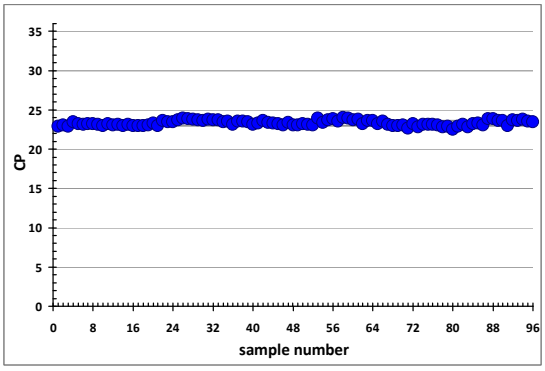

Figure 5 Reproducibility of DNA isolation. 96 identical samples (T7 DNA) were isolated as described above. T7 DNA was determined using a specific real-time PCR system. Highly reproducible results were achieved. Crossing point of 23.3+/-0.33, CV 1.42 %

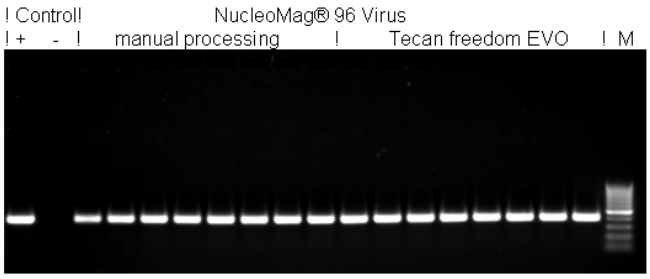

Figure 6 Amplified DNA of the T7 phage loaded onto a 1 % TAE gel, data generated with the magnetic separator from MACHEREY-NAGEL together with the robotic manipulator arm from Tecan

After DNA extraction of T7 phage DNA with the NucleoMag kit, a DNA fragment was amplified by PCR and the PCR fragments loaded onto a gel and analyzed. The high purity of the extracted DNA is demonstrated by the robust amplification of PCR fragments (Fig. 6).

To analyze the ability of the Freedom EVO to extract nucleic acids without cross-contamination, DNA extraction was performed in a checkerboard pattern (excluding positive controls) in a 96-well plate, and subsequent PCR reactions were run on the isolated DNA (Fig. 7). A PCR product could only be seen in wells where sample material had been present, clearly demonstrating that DNA was extracted without cross-contamination.

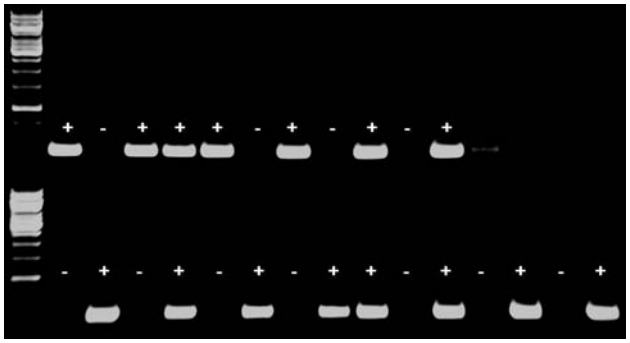

Figure 7 Amplified DNA of the T7 phage loaded onto a 1 % TAE gel, data generated with the magnetic separator from MACHEREY-NAGEL together with the robotic manipulator arm from Tecan

The data shown above emphasize the reproducibility of the application. However, the tests were performed using DNA/or RNA phage spike experiments. In order to demonstrate the performance of the system with a real example, the isolation of H12N5 viral RNA for veterinary testing is shown below (Fig. 8). A dilution series of a positive sample was prepared and viral RNA was isolated using the NucleoMag 96 RNA kit on a Freedom EVO workstation.

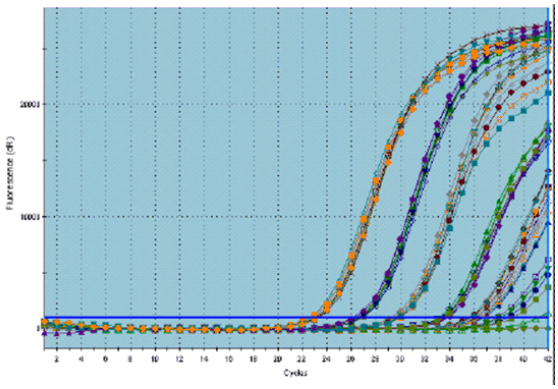

Figure 8 Isolation of viral RNA (AIV H12N5). A dilution series of a positive sample (cell culture supernatant) was prepared and isolated. Viral RNA was amplified by specific real-time RT-PCR assays for Influenza A Virus and the subtypes H5, H7 and N1. Homogenous and reproducible amplification of all samples was achieved.

Conclusion

Automation of the NucleoMag 96 Virus kit on a Freedom EVO workstation allows fast and reliable extraction of viral DNA in true walkaway mode. The combination of the kit and the workstation proved to be an exceptionally successful system for generating high quality NA in a consistent manner. For highest flexibility and changing laboratory needs, the Freedom EVO workstation can be equipped with a number of extension modules including an absorbance reader, storage modules and cooling devices. Talk to your local Tecan consultant to fine-tune your workstation according to your laboratory's needs.

Acknowledgements

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Further application notes

Tecan – MACHEREY-NAGEL
NucleoSpin 96 Virus
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Austria +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 21 28 98 63 33 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170
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