SAFE SAMPLE COLLECTION AND SCREENING IN VETERINARY DIAGNOSTICS.

Application note.



TECAN.

AUTOMATED PATHOGEN DNA/RNA PURIFICATION USING NUCLEOMAG® VET IN COMBINATION WITH NUCLEOPROTECT® VET ON THE DREAMPREP® NAP WORKSTATION

INTRODUCTION

Molecular detection tools are commonly used by veterinarians to screen for diseases in apparently healthy animals, diagnose infections, monitor disease progression and evaluate therapeutic responses. These techniques, which include qRT-PCR and next generation sequencing, rely on efficient nucleic acid purification as an essential step in the diagnostic workflow. Modern veterinary diagnostic laboratories must therefore be able to process, extract and subsequently analyze hundreds of samples every day – especially during disease outbreaks – making automation a necessary tool to keep up with the increasing demand.

In this application note, Tecan and MACHEREY-NAGEL demonstrate how the NucleoMag® VET kit can be used on the DreamPrep® NAP workstation to automatically extract pathogen DNA/RNA from blood and swab samples collected using NucleoProtect® VET.

- NucleoMag VET kit is an automation-friendly magnetic bead-based kit for extraction of viral RNA/DNA and bacterial DNA from various sample sources, such as serum, plasma, blood, tissue, feces, swabs or other cell-free biological fluids.
- NucleoProtect VET Swab and Blood Tubes enable safe collection of swab and blood samples, improving the sensitivity of molecular test results due to their reliable DNA/RNA stabilization and powerful sample inactivation.
- The DreamPrep NAP system allows full workflow automation, providing a significantly improved throughput and hands-free time for a wide variety of sample types.
- FluentControl™ software and TouchTools™ visual commands offer a user-friendly interface, reducing the need for training.

MATERIALS AND METHODS

The NucleoMag VET kit, together with the DreamPrep NAP workstation, was used for the automated extraction of various model viruses, as well as spiked viral RNA and DNA. The DreamPrep NAP platform – based on the Fluent® 480 Automation Workstation – is specifically configured for nucleic acid nucleic acid processing workflows, using magnetic bead-based extraction in combination with FluentControl™ GX Assurance Software (Figure 1).

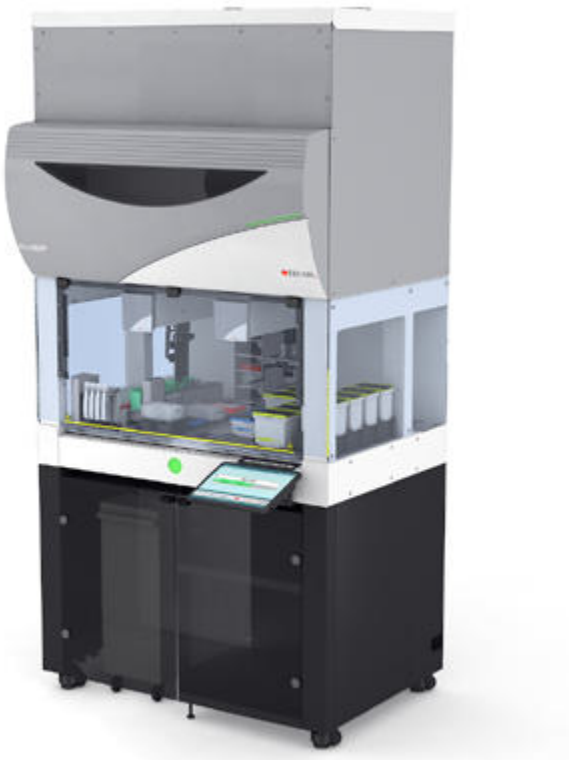

Figure 1: Representation of the DreamPrep NAP workstation, including integrated Quant/Norm with the Infinite® 200 Pro M Nano+.

The instrument is equipped with an Air Flexible Channel Arm™, a Robotic Gripper Arm™, Fluent ID™ and a handheld barcode scanner (Honeywell) for sample and reagent recording, a BioShake™ D30-T elm (QInstruments) for heating and shaking; and a NucleoMag SEP Magnetic Separator (MACHEREY-NAGEL). For quantification and quality control of the extracted nucleic acid the add-on module Infinite® 200 PRO reader can be integrated directly into the worktable for direct nucleic acid quantification using UV absorbance-based measurements without any sample loss (Figure 2). 13 mm tube racks were selected for compatibility with NucleoProtect VET Swab and Blood Tubes. Filtered, conductive 200 and 1,000 µl FCA tips were used for all experiments.

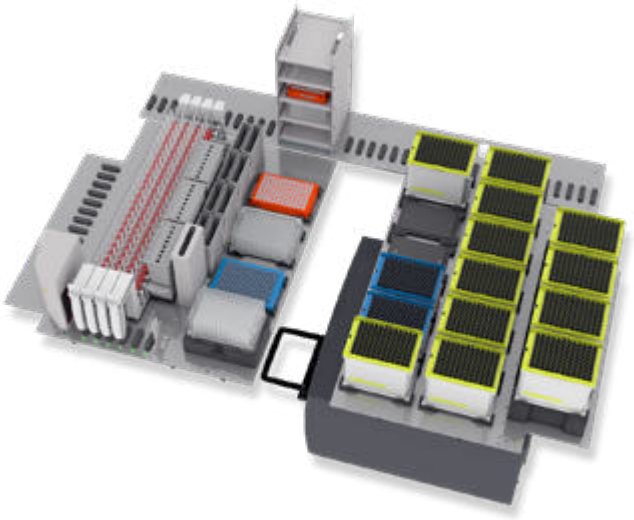

Figure 2: DreamPrep NAP featuring MACHEREY-NAGEL worktable configuration.

Nucleic acid extraction from virtually all veterinary sample types was performed using the NucleoMag VET kit (Table 1).

NucleoMag VET	
Technology	Magnetic beads
Sample material	200 µl animal whole blood 5-10 mg tissue, eg. ear notches 200 µl plasma 200 µl serum 200 µl milk 200 µl swab washes or other cell-free biological fluids NucleoProtect VET collected blood or swab samples Fecal samples Chewing cords Insects (eg. honey bees) Pollen
Target molecules	Viral RNA/DNA and microbial DNA
Fragment size	300 bp to ~50 kbp
Elution volume	50-200 µl

Table 1: NucleoMag VET kit product description.

The tailored protocol can be used with variable sample numbers, processing 1-96 samples per run. All samples were collected using the NucleoProtect VET sample collection tubes for single-step storage and transport, offering reliable DNA/RNA stabilization capacity and powerful inactivation of high risk viruses (Table 2). The integrated Fluent ID module enabled automated sample tracking and barcode reading of NucleoProtect VET Swab Tubes and NucleoProtect VET Blood Tubes (Figure 3).



Figure 3: Both NucleoProtect VET Swab Tubes and NucleoProtect VET Blood Tubes come with double barcode labels with a peel-off section. The peel-off part is self-adhesive, and can be transferred to sample request forms, allowing safe and secure sample tracking. The barcodes on NucleoProtect VET sampling tubes can be used for automated sample registration on the DreamPrep NAP using the Fluent ID barcode scanner.

NucleoProtect VET sampling formats	
NucleoProtect VET Blood Tube	Blood collection tube prefilled with 4.0 ml of NucleoProtect VET reagent
NucleoProtect VET Swab Tube	Screw cap swab collection tube prefilled with 1.5 ml NucleoProtect VET reagent, including sterile, individually packed flocked swabs and collection tube caps that feature integral swab capture
NucleoProtect VET reagent	Bottled NucleoProtect VET reagent

Table 2: NucleoProtect VET sample formats.

The quality of the nucleic acid sample extractions was verified through qPCR performed using the Applied Biosystems® AgPath-ID™ One-Step RT-PCR Reagents (Thermo Fisher Scientific) for RNA viruses, or the QuantiTect® Multiplex PCR Kit (Qiagen) for DNA viruses on a CFX96 Touch Real-Time PCR System (BioRad). Spiked DNA and RNA were detected using the Meridian Bioscience SensiFAST™ Probe Lo-Rox kit (DNA) or SensiFAST Probe Lo-Rox One-Step kit (RNA) on an Applied Biosystems 7500 Real-Time PCR System.

RESULTS AND DATA ANALYSIS

NucleoProtect VET tubes reliably preserve nucleic acids

The NucleoProtect VET reagent consists of a non-hazardous, proprietary formula that destroys viral envelopes and denatures capsid proteins, reducing the virulent nature of the samples while maintaining the integrity of viral nucleic acids.

To prove this, bovine saliva was spiked with low, medium and high viral titers of the canine distemper virus (CDV; (-)ssRNA), porcine enterovirus (PEV; (+) ssRNA), bluetongue virus (BTV serotype 8; dsRNA) or the vaccinia virus (POX; dsDNA). Spiked saliva samples were collected with NucleoProtect VET Swab Tubes and stored according to the indicated storage conditions (T_0 = 1 hour at room temperature; T_7 = 7 days at room temperature). After nucleic acid purification and subsequent analysis via qPCR, it was clear that nucleic acids were efficiently preserved independent of virus species, storage conditions or viral titer (Figure 4).

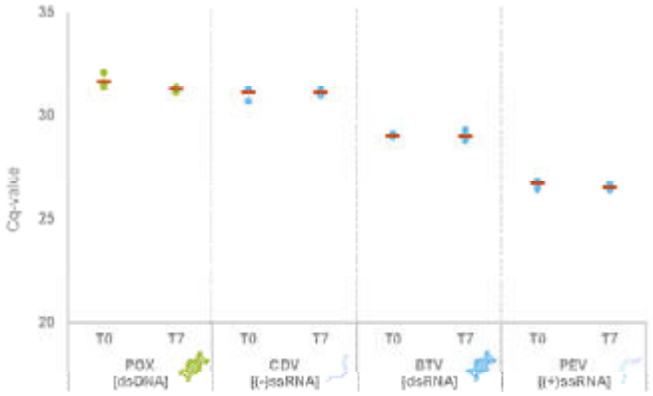

Figure 4: NucleoProtect VET Swab Tubes reliably preserve nucleic acids for all viral titer concentrations and viral genome structures. Sample barcodes were consistently tracked by the Fluent ID, and samples were purified in a reproducible manner (n=4).

The same bovine saliva was spiked in a 1:10 serial dilution of porcine enterovirus (PEV; (+)ssRNA) and samples were stored for 24 hours at room temperature before being purified using the DreamPrep NAP workstation and analyzed by qPCR. The data shows almost perfect linearity for the purified target RNA – with an R^2 value of 0.9999 – demonstrating the robustness and reproducibility of the nucleic acid stabilization and automated extraction workflow (Figure 5).

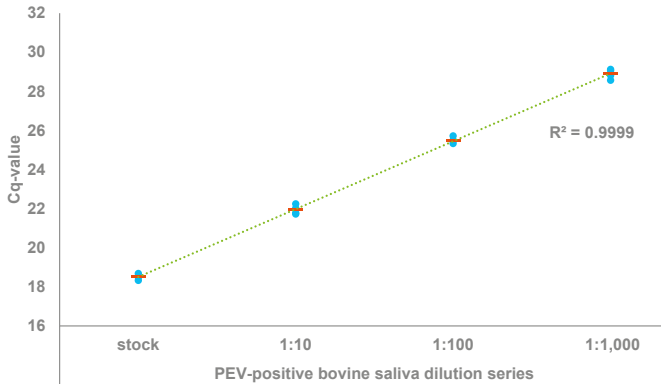

Figure 5: High reliability of veterinary test results independent of viral titer load. The purified target RNA shows almost perfect linearity (R^2 = 0.9999).

Extraction of protected samples results in more consistent C_T values in qPCR workflows

Samples spiked with T7 bacteriophage DNA and MS2 bacteriophage RNA were collected with NucleoProtect VET Blood Tubes and EDTA tubes. Nucleic acids were then extracted using the automated DreamPrep NAP platform and analyzed via qPCR. The degradation of DNA was compared between the two tube types and the results showed that spiked MS2 RNA in standard EDTA tubes was completely degraded after a short time and was no longer detectable. Meanwhile, the degradation of spiked DNA in EDTA tubes was less notable and the target molecules were still measurable, even after a storage period of seven days. The data also indicated that EDTA allowed detection of lower levels of DNA, but that might not be a true reflection, because the dilution of the NucleoProtect samples was not taken into consideration. Additionally, it was shown that NucleoProtect VET-collected blood samples, unlike those stored in EDTA tubes, allowed reliable detection of all target molecules under various storage conditions (Figure 6). This data indicates that the nucleic acid stabilization provided by NucleoProtect VET can significantly increase the reproducibility and reliability of molecular veterinary test results.

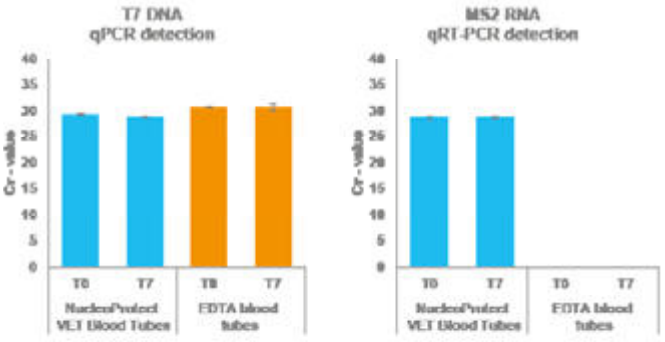

Figure 6: Nucleic acid stabilization with NucleoProtect VET Blood Tubes significantly increases the reliability of molecular veterinary test results. NucleoProtect VET-collected blood samples allowed reliable detection of T7 bacteriophage DNA and MS2 bacteriophage RNA under various storage conditions.

SUMMARY

MACHEREY-NAGEL and Tecan have collaborated to demonstrate a tailored workflow that can meet the demands of high throughput veterinary diagnostic laboratories. NucleoProtect VET Blood Tubes and NucleoProtect VET Swab Tubes can be used to safely collect samples on farms and in the wild and are suitable for use with the NucleoMag VET extraction kit automated on the DreamPrep NAP workstation. All steps, from sample scanning to elution of the final ultra-pure extract, can be handled automatically by the DreamPrep NAP platform, thanks to Fluent ID and the prelabeled barcodes on the NucleoProtect VET tubes. This set-up also allows automatic sample registration and tracking. The magnetic bead-based extraction and sample protection technology from MACHEREY-NAGEL, together with Tecan's DreamPrep NAP platform, offers a walkaway workflow for the reliable automated extraction of pathogen DNA/RNA, which can be used for the diagnosis and prevention of animal diseases.

- Tailored protocols for various sample types and sample volumes
- Flexible kit that can purify DNA/RNA in all types of veterinary samples
- Easy and safe workflow using the NucleoProtect VET barcodes and the Fluent ID system for sample registration and tracking
- Reliable test results through NucleoProtect VET DNA/RNA stabilization
- NucleoProtect VET protects the user by effectively inactivating infectious viruses
- Automation reduces hands-on time, enabling higher throughput

ACKNOWLEDGEMENTS

This protocol was developed by MACHEREY-NAGEL application scientists and is intended for research use only. Users are responsible for assessing the suitability of the protocol for their application.

LEARN MORE

To learn more about Tecan® nucleic acid purification solutions, contact your sales representative or visit www.tecan.com/NAP.

To learn more about MACHEREY-NAGEL's NucleoProtect VET stabilization and inactivation reagent, or other solutions for veterinary diagnostic workflows, please visit www.mn-net.com/animalhealth.
Customized barcoding of NucleoProtect VET Swab or Blood Tubes is available upon request.

ABOUT THE AUTHORS



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Dr. Ansgar Flammersfeld is a former product manager in the bioanalysis department at MACHEREY-NAGEL. He studied molecular and applied biotechnology at RWTH Aachen University. His PhD at the Institute for Cellular and Applied Infection Biology focused on understanding the molecular mechanisms of the human malarial parasite Plasmodium falciparum. He joined MACHEREY NAGEL from 2020-2024 , and mainly worked in the field of applied genomics in the context of high throughput nucleic acid purification and automation systems.

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