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APPLICATION NOTE No. 381

Purification of viral nucleic acids from Blood, Tissue and Chewing cords NucleoMag® VET Kit on the ep*Motion*® 5075t/m

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Abstract

Purification of nucleic acids in veterinary diagnostics is routinely performed in a variety of research laboratories with a growing demand for automation. The extracted RNA or DNA is later used for the detection of pathogens, such as viruses, bacteria or parasites, in veterinary samples. Automation significantly facilitates the purification especially for potentially hazardous sample material and for a broad range of sample material like animal whole blood, serum, feces, ear notches, tissue or swabs. The NucleoMag® VET Kit from MACHEREY-NAGEL is here adapted to the ep*Motion*® 5075t/m. The combination of the ep*Motion* 5075t/m and the extraction kit, allows an efficient walk away purification of DNA and RNA in less than 120 minutes.

Introduction

The procedure of the NucleoMag® VET kit on ep*Motion* 5075t/m is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. The prelysis procedure is sample-specific and can differ between sample materials. The NucleoMag® VET kit is suitable for a broad variety of starting materials, such as animal whole blood, serum, feces, urine, ear notches, tissue, salvia or swabs. The ep*Motion* 5075 allows purifying all this samples fast and comfortable. Subsequently the samples were treated with Lysis buffer VL and the released nucleic acids are bound to the paramagnetic beads under appropriate binding conditions. The contaminants are removed through two washing steps with wash buffer VEW 1 and VEW 2. Salts are removed with an additional wash step using 80% ethanol. The purified nucleic acids are eluted and can be used directly as a template for qPCR, next generation sequencing, or any kind of enzymatic reactions.

A purification process with 96 samples with re-use tips for the wash steps requires 240 x 1000 µl tips and 98 x 300 µl tips. This application note describes the configuration and preparation of the ep*Motion* 5075t/m to automate this kit.

Materials and Methods

Required labware

> Eppendorf ep*Motion* 5075t or 5075m
> Dispensing Tool TM 1000-8
> Dispensing Tool TM 300-8
> Reservoir Rack
> Reservoirs 30 mL / Reservoirs 100 mL
> Reservoir 400 mL
> NucleoMag® Sep (Magnetic separator)
> NucleoMag® VET Kit

Required consumables

> epT.I.P.S® Motion 1000 µL with filter
> epT.I.P.S Motion 300 µL with filter
> Eppendorf Deepwell Plate 96/2000 µL
> Eppendorf Microplate 96V

Samples

Blood from cattle and pig, tissue (spleen and heart from pig), samples from chewing cords and a dilution series of bacteriophage MS2-RNA

Method

This protocol is developed to process up to 96 samples in parallel on the epMotion 5075m or 5075t workstation.
This kit is suitable for up to 200 µL of blood, 10 to 30 mg tissue or 200 µL of chewing cord samples. The samples require different prelysis steps. For example blood could be diluted 1:1 with PBS buffer, but could be also used directly. For saliva from chewing cords it is the same procedure.
Tissue samples were homogenized with 400 µL PBS, 20 µL Proteinase K and 20 µL DTT (Dithiothreitol) with a 3 to 4 mm steal bead for 3 min. at maximum speed in a Tissue Lyser or something similar. This is followed by a mixing step at 56°C for 2 h. A filtration or centrifugation step clears the prelysate. After the prelysis steps, 200 µL of the cleared solution is prefilled into each well of the separation plate. All subsequent steps are automated on the ep*Motion* and will be carried out in this plate. This includes dispensing of buffers and beads, removal of the supernatants as well as transport, temperature incubation and mixing steps.
After the prelysis step, the lysis buffer VL is added. After a mixing and heating step, magnetic beads and binding buffer are added. During a mixing and incubation step, the viral DNA/RNA is bound to the magnetic beads. Beads are separated on a magnetic plate adapter and the supernatant is removed. Unspecific bound contaminants are removed by several washing steps with wash buffers VEW1 and VEW2. Remaining salts are removed with an additional wash step using 80% ethanol. After the last washing step, residual ethanol is removed in a drying step of 10 min. at 56°C.

In a last step, the eluate is transferred to a dedicated elution plate. A purification process with 96 samples with re-use tip function for the wash steps requires 240 x 1000µl tips and 98 x 300 µl tips and takes less than 2 h.
For the method the following positions of the worktable are occupied:

Position	Labware
A2	300 µL filtertips
A3	300 µL filtertips
TMX	Separation Plate (Lysed samples)
B1	1000 µL filtertips
B2	1000 µL filtertips
B3	1000 µL filtertips
C2	Liquid Waste (400 mL reservoir)
C3	NucleoMag® Sep
C4	Reagent reservoirs
C5	Elution Plate

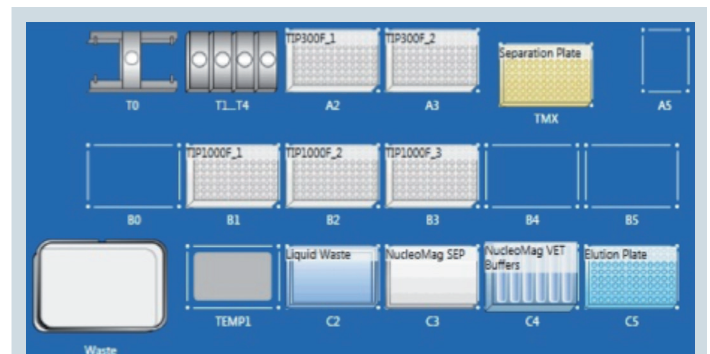

Figure 1: Worktable allocation

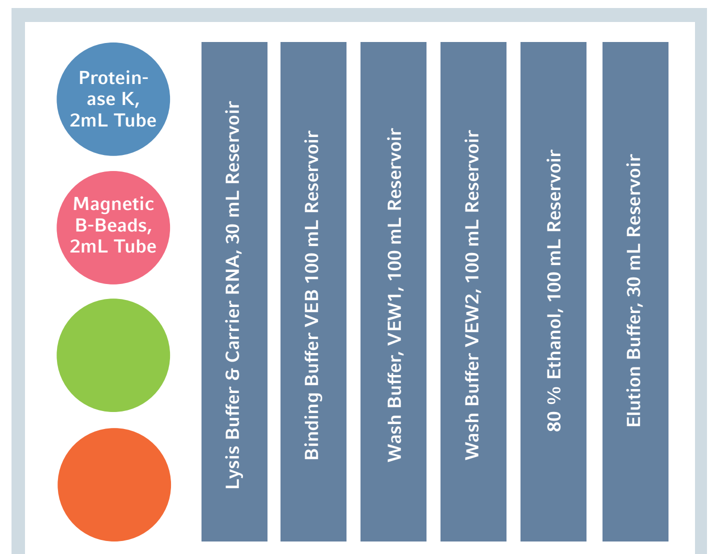

Figure 2: ReservoirRack

Results and Discussion

The NucleoMag® VET kit is designed for the isolation of total viral RNA and DNA. Purification results from different veterinary material, like blood, tissue or chewing cord are shown. Yield and purity were determined by UV spectroscopy with Synergy™ HT Multi-detection microplate reader (Biotek®). Furthermore a qPCR with SensiFast®Probe Lo-Rox Kit (Bioline) on an Applied Biosystems® 7500 was used to check for the absence of inhibitors.

Yield and Purity

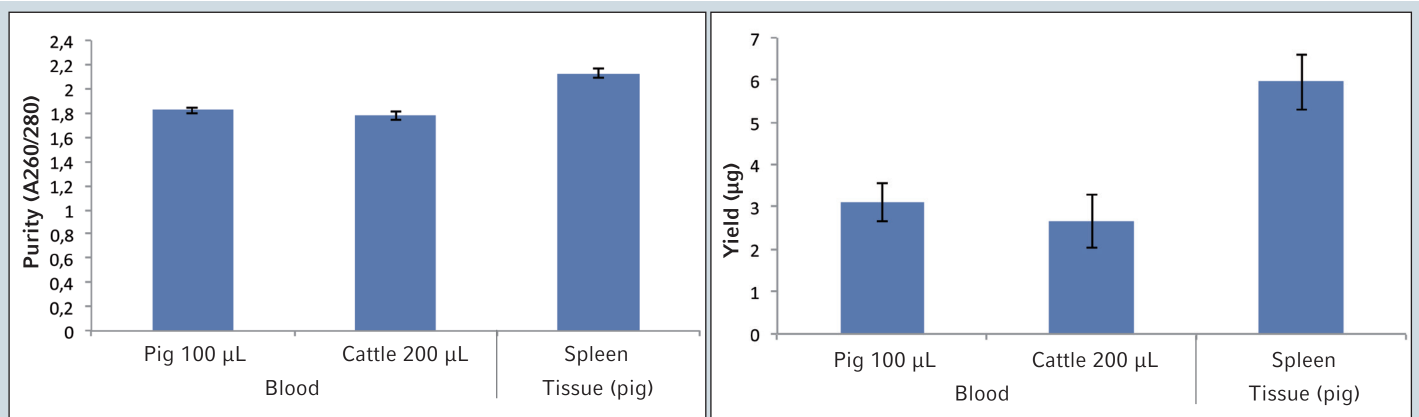

Figure 3 and 4: Yield and Purity of simultaneous purified DNA/RNA from blood of pigs and cattle and from tissue samples (spleen) with Magnetic Beads. The purity and the yield were determined by UV spectroscopy.

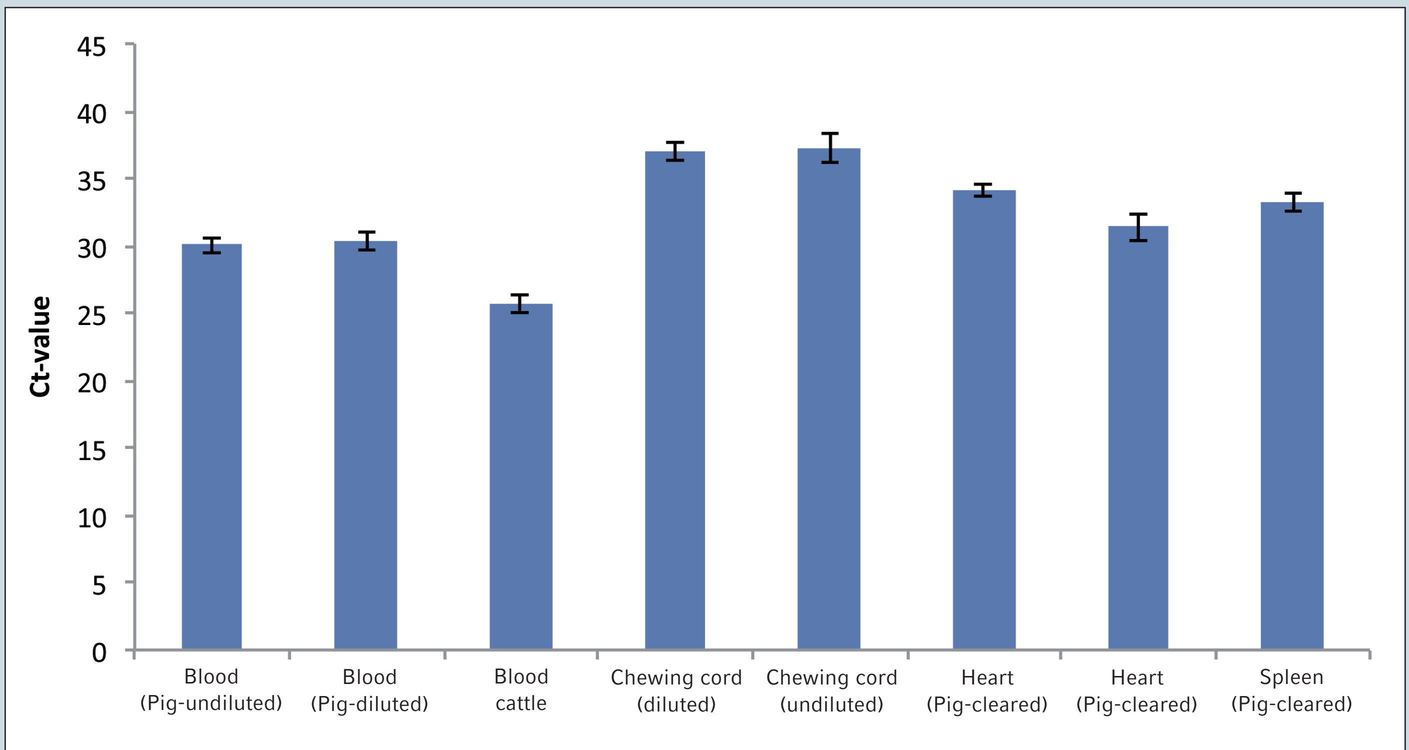

Figure 5: Ct-values of DNA/RNA from blood, chewing cord and tissue samples. Quantitative PCR-analysis was performed with 4 µL of eluate using a Taqman® probe for beta-actin and 250 bp amplicon size.

MS2-RNA recovery:
To demostrate the sensitivity four different dilutions of the bacteriophage MS2-RNA were purified with the here described method. The MS2-RNA is diluted in RNase-free water.The recovery is shown in comparison to the input via quantitative RT-PCR.

Not diluted	4000 pg
1:10	400 pg
1:100	40 pg
1:1000	4 pg

For comparison the appropriate amount of template was applied in a quantitative RT-PCR.

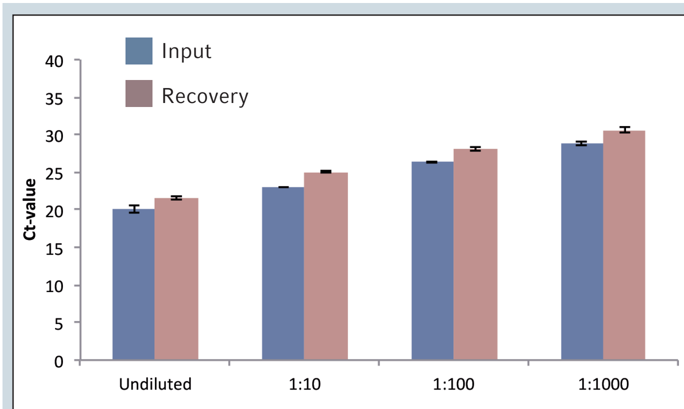

Figure 6: Ct-values of four different MS2-RNA-dilutions (4000 pg, 400 pg, 40 pg and 4 pg) in comparison to the input. MS2 amplicon size is about 400 bp. 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe.

Cross-contamination
The absence of cross-contamination was verified using a checkerboard pattern for extraction. No PCR amplification could be observed in eluates from negative controls.
4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe.

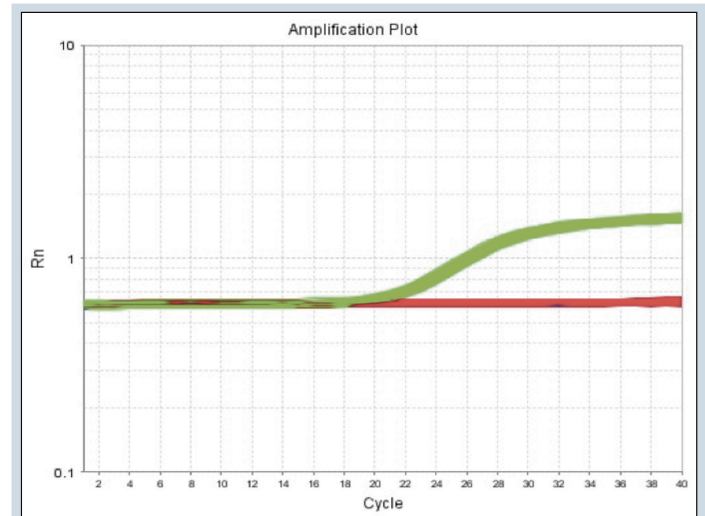

Figure 7: Cross-contamination check with MS2-RNA (checkerboard pattern) as sample material. No PCR amplification could be observed in eluates from negative controls. MS2 amplicon size is about 200 bp, 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe (96 samples).

Conclusion

The above results show that the combination of the NucleoMag® VET kit and the ep*Motion* 5075m/t reliably delivers high yields and of high quality DNA/RNA - from different sample material, like blood, tissue or chewing ropes. No cross contamination is detectable. The purified DNA/RNA is suitable for a full range of downstream methods.

The results from the qPCR, purity and yield show the good performance of the described procedure. The total time to process 96 samples is 110 minutes.
The use of Eppendorf SafeRack along with the re-use function, have a positive impact on the cost per sample.

Ordering information

Description	Order no. International
ep*Motion*® 5075t	5075 000.302
ep*Motion*® 5075m	5075 000.305
ReservoirRack	5075 754.002
TM 1000-8 Dispensing tool	5280 000.258
TM 300-8 Dispensing tool	5280 000.231
epT.I.P.S® Motion 1000 µL SafeRack with filter	0030 014.650
epT.I.P.S® Motion 300 µL with filter	0030 014.456
Reservoir 30 mL	0030 126.505
Reservoir 100 mL	0030 126.513
Reservoir 400 mL	5075 751.364
Deepwell Plate 96/2000 µL	0030 501.306

MACHEREY-NAGEL	
NucleoMag® VET	REF 744200.1/4
NucleoMag® SEP	REF 744900

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