



Nucleic acid purification from veterinary samples

MN solutions from single to high-throughput applications



Demands on veterinary testing

Challenges

- Diverse starting material
- Various species
- Difficult-to-lyse pathogens
- Low amount of nucleic acids
- Variable number of samples

MN-Solutions

- One-for-all solution
- Strong lysis buffer
- Efficient recovery
- Single spin & HTP



Single spin versus HTP – MN solutions



NucleoSpin® Virus

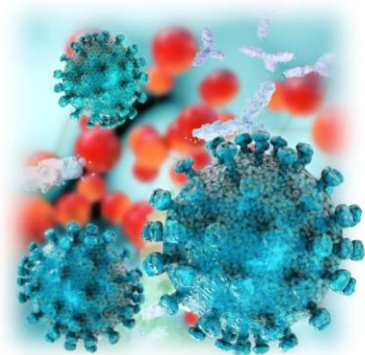


NucleoMag® Vet



NucleoSpin® Virus

Feature	NucleoSpin® Virus
Technology	Silica membrane
Format	Mini Spin columns
Sample Volume	Up to 400 µL
Lysis	Liquid Proteinase K
Fragment Size	100 bp – 50 kbp
Elution Volume	30 µL
Binding Capacity	25 µg



Evaluation of MN products

FRIEDRICH-LOEFFLER-INSTITUT

FLI

Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health



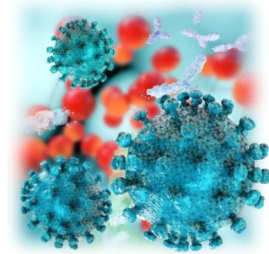
Bundesministerium
für Ernährung
und Landwirtschaft

German Federal authority

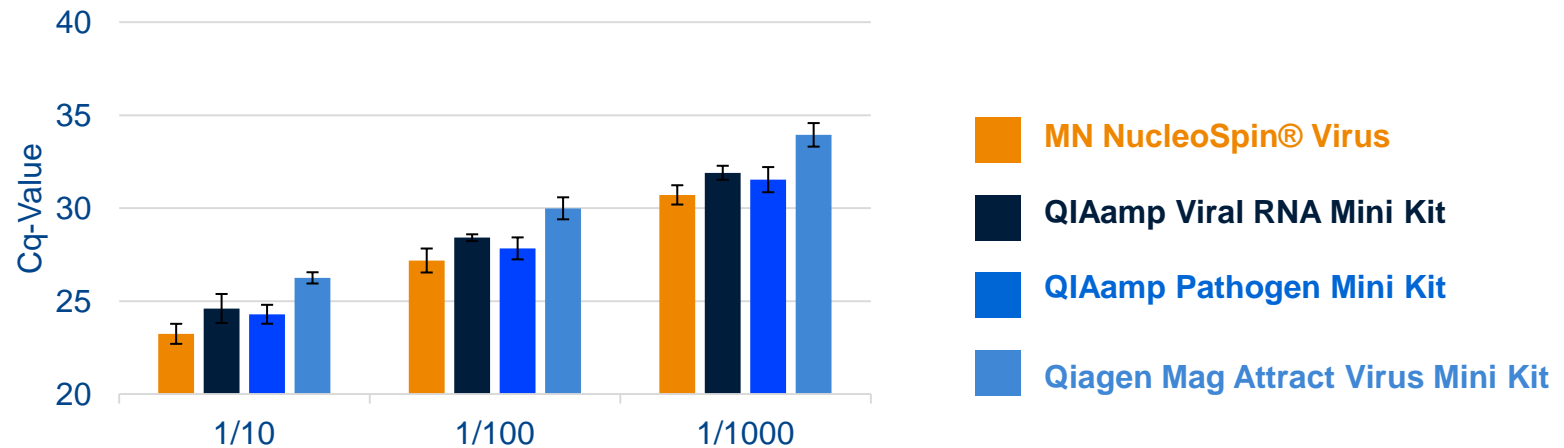
National Admissions Office for Veterinary Infection Diagnostics

CSF detection with NucleoSpin® Virus

Serum
ssRNA virus



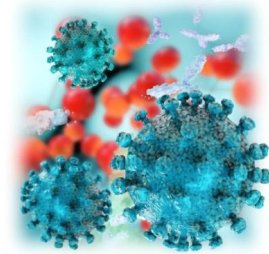
Classical Swine Fever



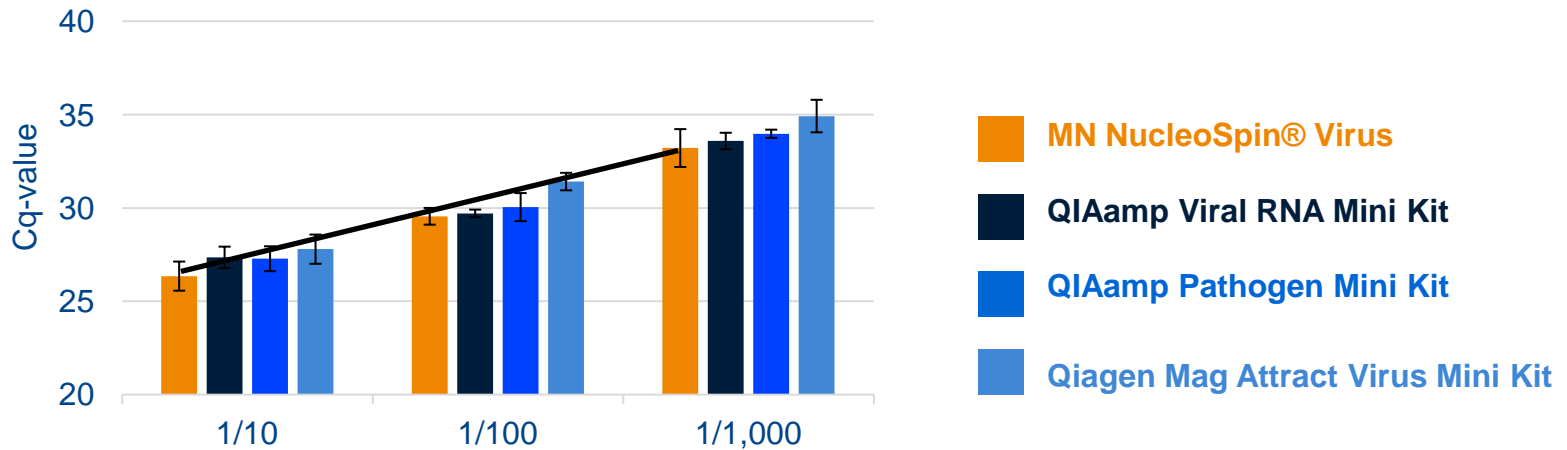
Data kindly provided by | **FLI** |

BTV detection with NucleoSpin® Virus

Blood
dsRNA virus



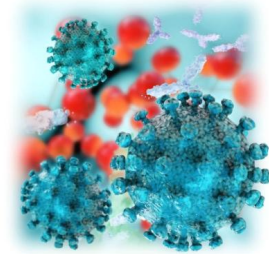
Blue Tongue Virus



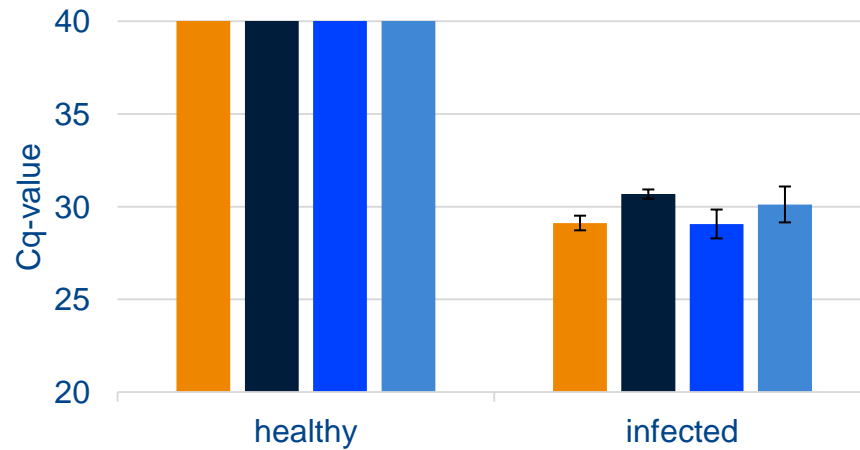
Data kindly provided by | **FLI** |

pCV-2 detection with NucleoSpin® Virus

Tissue
ssDNA virus



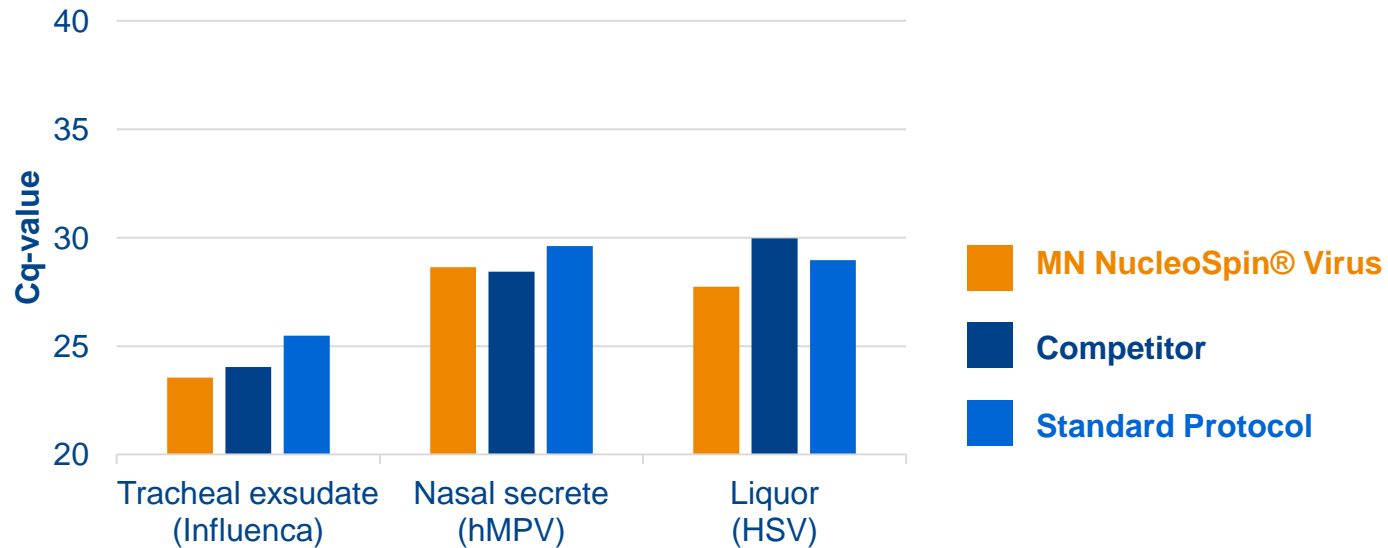
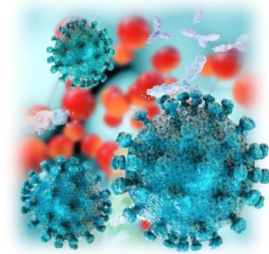
Porcine circovirus



-  MN NucleoSpin® Virus
-  QIAamp Viral RNA Mini Kit
-  QIAamp Pathogen Mini Kit
-  Qiagen Mag Attract Virus Mini Kit

Data kindly provided by | **FLI** |

Virus detection with NucleoSpin® Virus

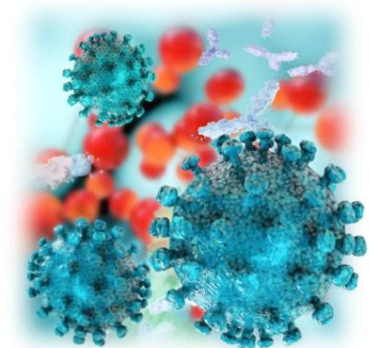


Data kindly provided by labor)krone

NucleoSpin® Virus

Customer values

- Tested by independent federal and non-federal institutions
- Suitable for liquid body fluids and tissue
- Efficient recovery of viral nucleic acids
- Suitable for human and animal samples
- Up to 400 µL starting volume
- Easy Handling (E.g., Liquid Proteinase K included)



Single spin versus HTP – MN solutions



NucleoSpin® Virus



NucleoMag® Vet



MACHEREY-NAGEL - cooperations

Thermo
SCIENTIFIC



TECAN.



HAMILTON



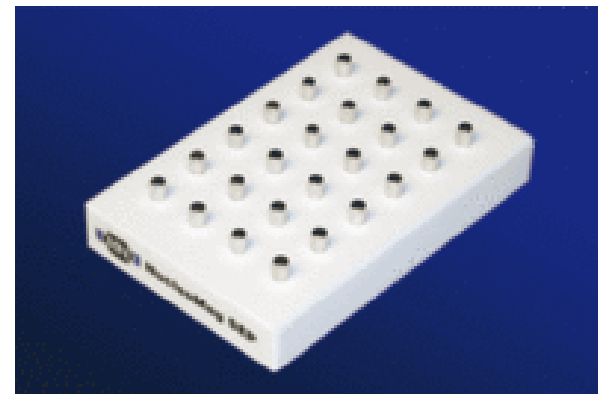
eppendorf



High-throughput instrumentation with MN

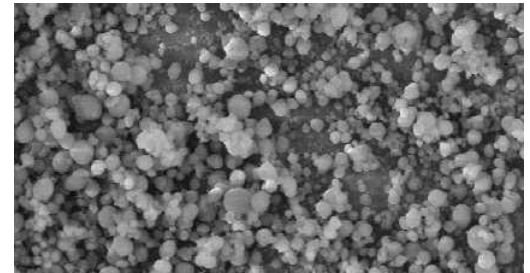
NucleoMag provides flexibility

- Processing of an adjustable number of samples per run
- Protocols can be adapted to sample volume
- Manual or automated (Magnetic separator)
- One-tube-reaction possible: no cross-contamination
- Small elution volumes $\geq 50 \mu\text{L}$



NucleoMag® Beads - optimized for automated use

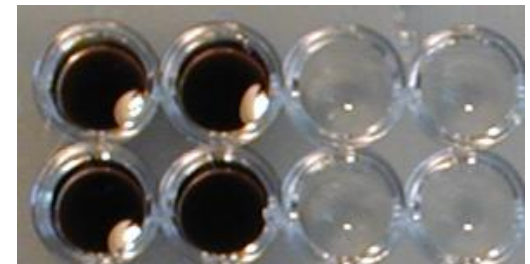
- polymer particle
- no silica cover
- size: 1 – 3 μm
 - large bead surface \rightarrow high binding capacity
- super paramagnetic
 - fast separation, high reactivity
 - no clumping
 - low risk of bead carry-over



Beads



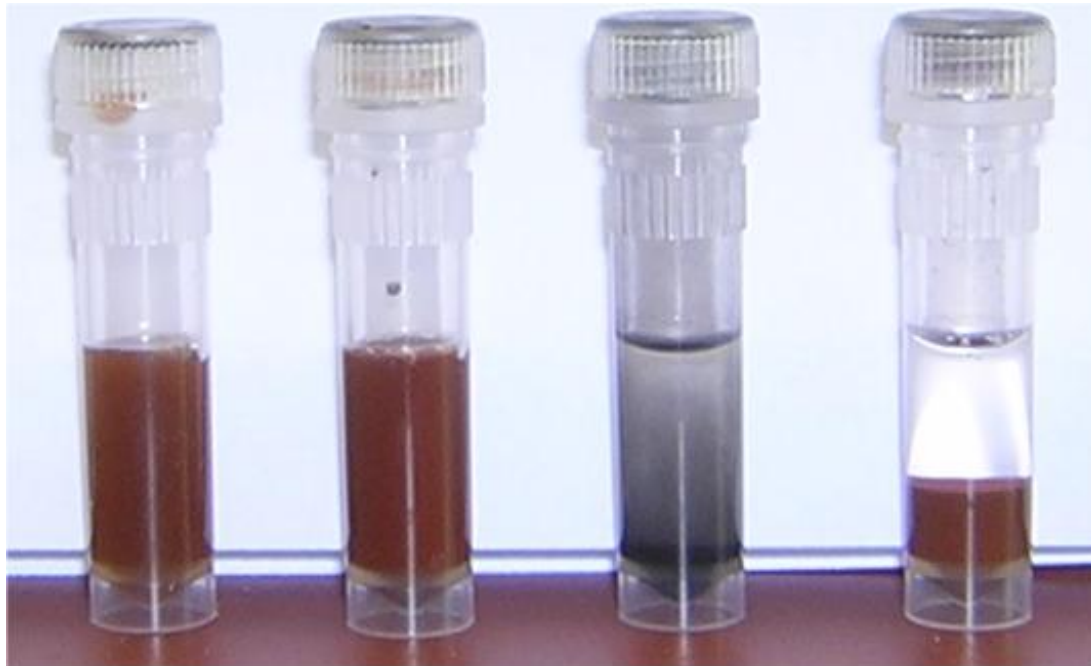
Beads on magnet



Beads off magnet

NucleoMag® Beads

Magnetic beads sedimentation
(10 min after complete resuspension)



Agencourt

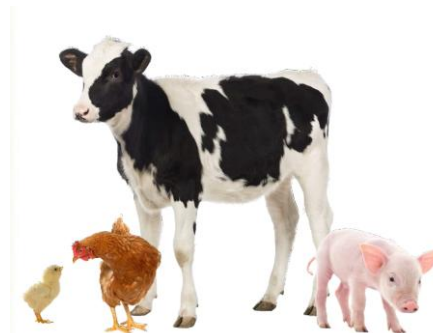
MN

Roche

Qiagen

NucleoMag® VET

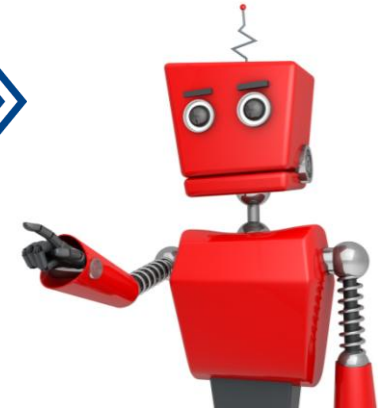
Feature	NucleoMag® VET
Technology	Magnetic Beads
Format	Highly reactive superparamagnetic beads
Processing	Manual or automated
Sample Volume	Up to 200 µL/30 mg tissue
Fragment Size	300 bp – 50 kbp
Elution Volume	50 µL
Preparation Time	96 preps/45 min
Binding Capacity	0.4 µg/µl beads





NucleoMag® VET - Automation

Available Scripts:



Hamilton



STARlet

Tecan



Freedom EVO

Eppendorf



epMotion

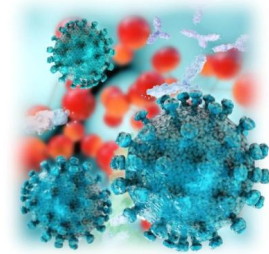
Thermo



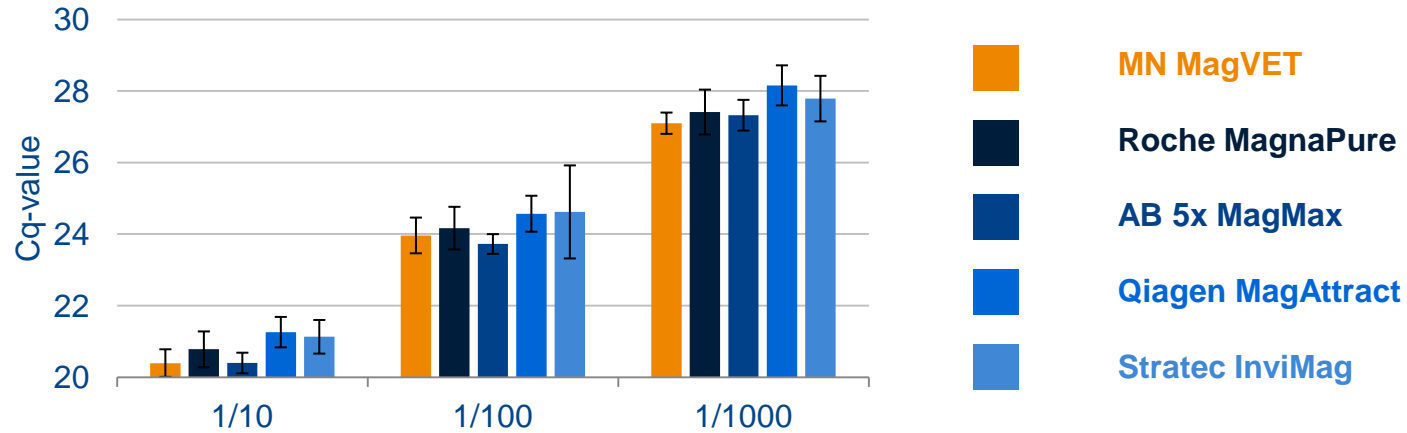
KingFisher

BTV detection with NucleoMag® VET

Blood
ssRNA virus



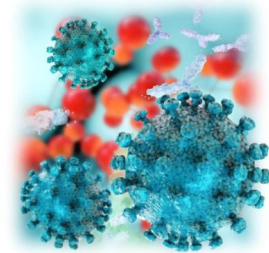
Blue Tongue Virus



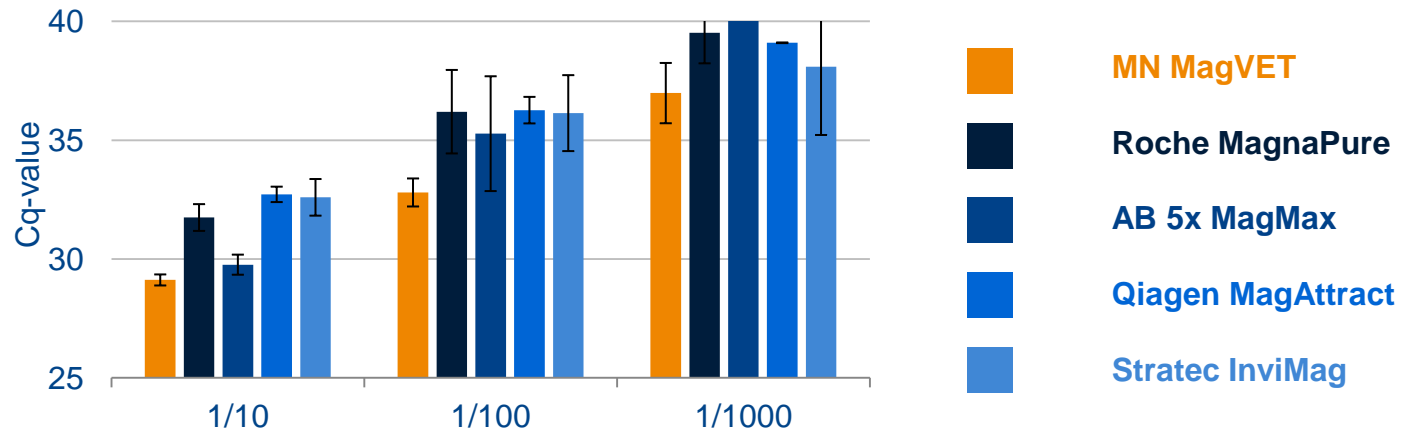
Data kindly provided by | **FLI** |

SBV detection with NucleoMag® VET

Serum
ssRNA virus



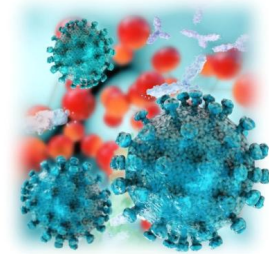
Schmallenberg Virus



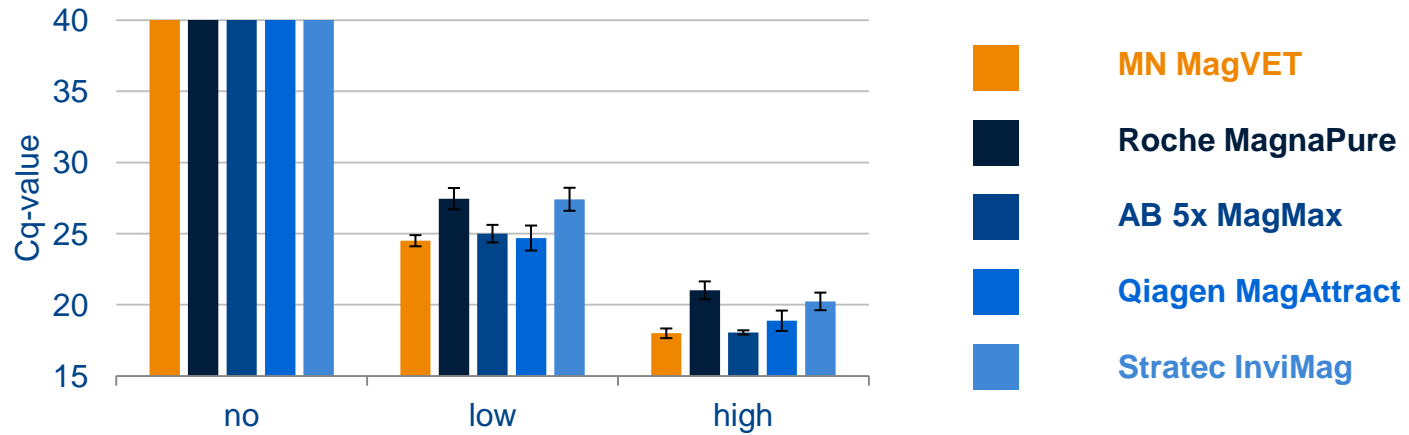
Data kindly provided by | **FLI** |

pCV-2 detection with NucleoMag® VET

Tissue
ssDNA virus



Porcine Circovirus



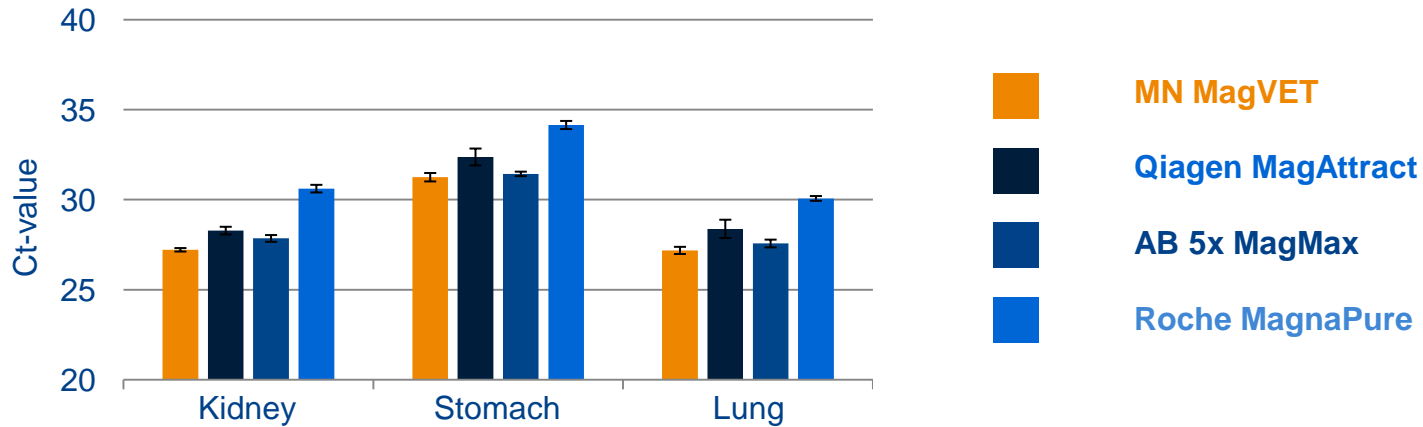
Data kindly provided by | **FLI** |

Chlamydia detection with NucleoMag® VET

Tissue
Bacteria (-)



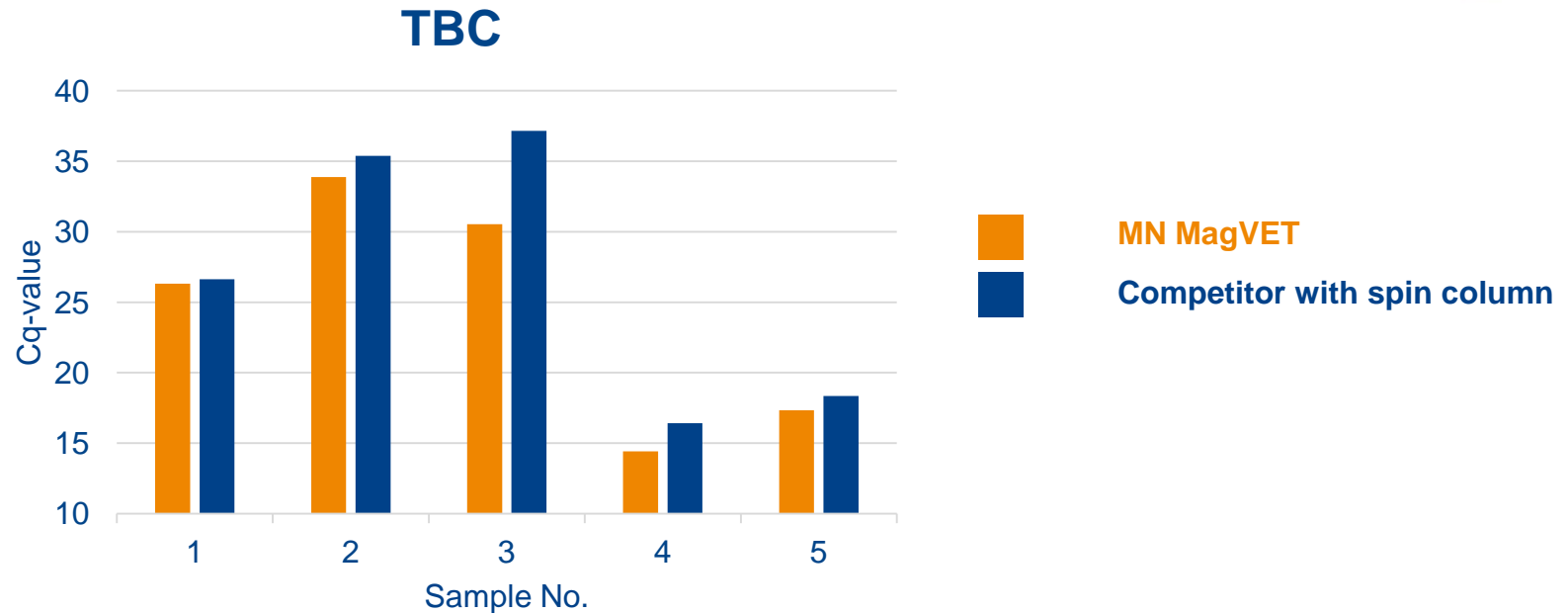
Chlamydiae



Data kindly provided by | **FLI** |

Detection of bacterial nucleic acids

Stool
Bacteria (+)

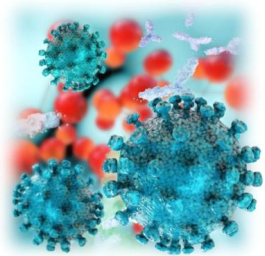


Data kindly provided by labor)krone

NucleoMag® VET Kit

Customer values

- Isolation of viral and bacterial DNA/RNA from difficult tissues
- Detection of nucleic acids at low amounts
- Already adapted on various automated platforms
- Flexibility regarding sample type, number and volume
- Traceability of sample processing
- Suitable for veterinary and human samples



Do you need help?

www.mn-net.com/Bio-HTP

Application Note

Plant genomics accelerated

Fast and reliable DNA extraction from plants with NucleoMag® 96 F NAGEL on a Freedom EVO® platform.

TECAN

Introduction

DNA extraction from plant material is an integral step both in plant research and in food analysis. Plant research is often geared towards crop improvement, focusing on yield, resistance to pathogens and other stress factors, such as heat or drought. Common applications include TILLING (Targeting Induced Local Lesions IN Genomes) and the creation of genetically modified species, as well as traditional breeding technologies. In all cases, breeding success needs to be confirmed not only by phenotyping, but by genotyping as well, creating a need for high throughput genomic DNA extraction.

Similar needs apply to the analysis of plant material in food diagnostics where, for example, the presence or absence of genetic modifications need to be verified.

MACHEREY-NAGEL has developed the NucleoMag 96 Plant kit in order to fulfil the need for fast and homogeneous extraction of high quality DNA from a variety of plants and fungi. The magnetic bead-based extraction process delivers

high quality DNA in regard to scalability numbers. Tecan's efforts to provide a

After initial hom the workflow ca Freedom EVO contamination. Sample track consequently

Processing samples. To nucleic ac typically y material, compare



Automated Plasmid Purification on the Genomic STARlet

Flexible and efficient workstations for isolation of DNA have become more important in the past few years in many areas of life sciences, as sample preparation is a time-consuming step in genetic analysis. As a solution to this problem, HAMILTON and MACHEREY-NAGEL have designed and validated the Genomic STARlet for different applications, e.g. plasmid DNA purification. The flexible purification system is based on the liquid handling robot MICROLAB® STARlet suitable for use of the silica membrane based NucleoSpin® 8/96 Plasmid Purification Kits from MACHEREY-NAGEL.

System Requirements	Part Number	
Genomic STARlet, 8 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Classic Life Science Package	806300	
Genomic STARlet, 8 channels, CVS Vacuum Station, HAMILTON Heater Shaker, Classic Life Science Package	806370	
Labware Requirements, Kits	Kit	Part Number
NucleoSpin® 8 Plasmid	12 x 8	FA921.1
	60 x 8	FA921.5
NucleoSpin® 96 Plasmid	1 x 96	FA921.7
	4 x 96	FA921.4
	24 x 96	FA921.24

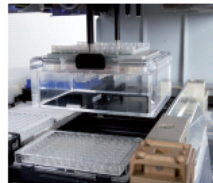


Figure 1: Transport of CVS vacuum manifold loaded with 8-well NucleoSpin® 8/96 Plasmid Binding strips on the adapter plate using the HAMILTON CO-RE Gripper.

LIFE SCIENCE ROBOTICS

Applications

Note 168 | March 2010

Automated RNA purification in 96-well plate and 8-well strip format from human cells or animal tissue using the MACHEREY-NAGEL NucleoSpin® 8/96 RNA kits on the epMotion® 5075 from Eppendorf

winning Risch, Thomas 20th, MACHEREY-NAGEL GmbH & Co. KG, D6910, Germany
 Michael Warrnath, Eppendorf AG, Hamburg, Germany

Abstract

Application note we describe the integration of the MACHEREY-NAGEL NucleoSpin® 8/96 RNA kits into the epMotion® 5075 VAC automated pipetting system. The NucleoSpin® 8/96 RNA kits are based on a well proven extraction based bead-wash procedure. Protocols for the epMotion 5075 VAC are available for medium scale of NucleoSpin 8/96 RNA kits on the epMotion 5075 VAC using the 96-well plate based extraction data for RNA isolation from mouse or pork tissue and human cells or for analysis on the Agilent 2100 Bioanalyzer and in qRT-PCR experiments.

total RNA from cells and tissue is a pre-requisite for gene expression or gene expression analysis. In addition, RNA has to be extracted with high structural integrity. The demands on RNA isolation are therefore high. Recent sample materials are robust, hard to pipette and easy to handle. The use of silica membrane purification kits on the epMotion® 5075 VAC using the well proven extraction based bead-wash procedure is a well proven method to isolate high quality RNA. In addition, the use of an optimized silica membrane allows RNA isolation from up to 2 x 10⁶ cells or 10-20 mg of tissue. Depending on the sample type and amount yields of up to 20-40 µg RNA can be achieved.

can readily be automated on liquid handling systems. The NucleoSpin RNA procedure is based on simple lysis using a lysis buffer containing active cleaning steps of silica supports the amount of RNA obtained from tissue samples remaining DNA is removed by an on-column DNase digestion step. In subsequent washing steps, proteins and other contaminants are removed. Finally the purified RNA is eluted in water and can be used for further downstream applications. The use of an optimized silica membrane allows RNA isolation from up to 2 x 10⁶ cells or 10-20 mg of tissue. Depending on the sample type and amount yields of up to 20-40 µg RNA can be achieved.

The purified RNA is suitable for use in several downstream applications, e.g. real-time qRT-PCR. The use of NucleoSpin RNA kits on the epMotion 5075 VAC automated pipetting system provides excellent results without the need for extensive programming, optimization and set-up time.

eppendorf

MN supports your HTP-implementation

Our expertise	Your benefit
Pioneers of DNA/RNA/protein purification	Well-thought-out solutions addressing your requirements
More than 15 years of HTP experience: <ul style="list-style-type: none">• Product and protocol development for all common applications• HTP adaption of existing products for all common platforms	MN meets your HTP product needs One-stop-shopping at MN
Customization of products and protocols	Highest flexibility
The MN R&D department is equipped with all common robot platforms	Direct support of your HTP-applications by MN <ul style="list-style-type: none">- Development of platform-specific protocols- Validation of MN methods- Trouble-shooting

Your MN OEM Team

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Thank you!



Stability data on NucleoMag VET

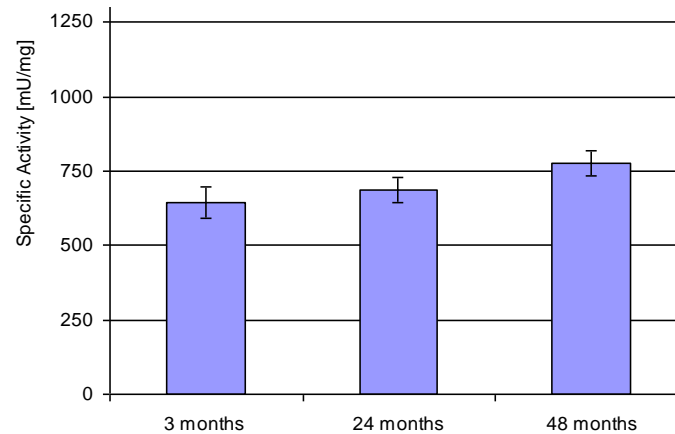
Stability of beads

	magnetic beads after storage		freshly produced magnetic beads	
	LOT	manufacturing	LOT	manufacturing
	508/001	August 2005	1007/001	July 2010
DNA yield, mean µg		4.00+/-0,08		3.75+/-0.12
A260/280, mean		1.75+/-0.02		1.81+/-0.04
	612/001	December 2006		
DNA yield, mean µg		3.93+/-0.08		
A260/280, mean		1.75+/-0.03		
	809/2008	September 2008		
DNA yield, mean µg		3,78+/-0.08		
A260/280, mean		1.78+/-0.04		

DNA yield of all stored bead lots is higher (>100 %) than DNA yield of the reference bead lot, 'DNA purity (A260/280 ratio) of the stored bead lots of 1.75 +/-0.02, 1.75+/-0.03 and 1.78 +/-0.04 fulfill the release specification.

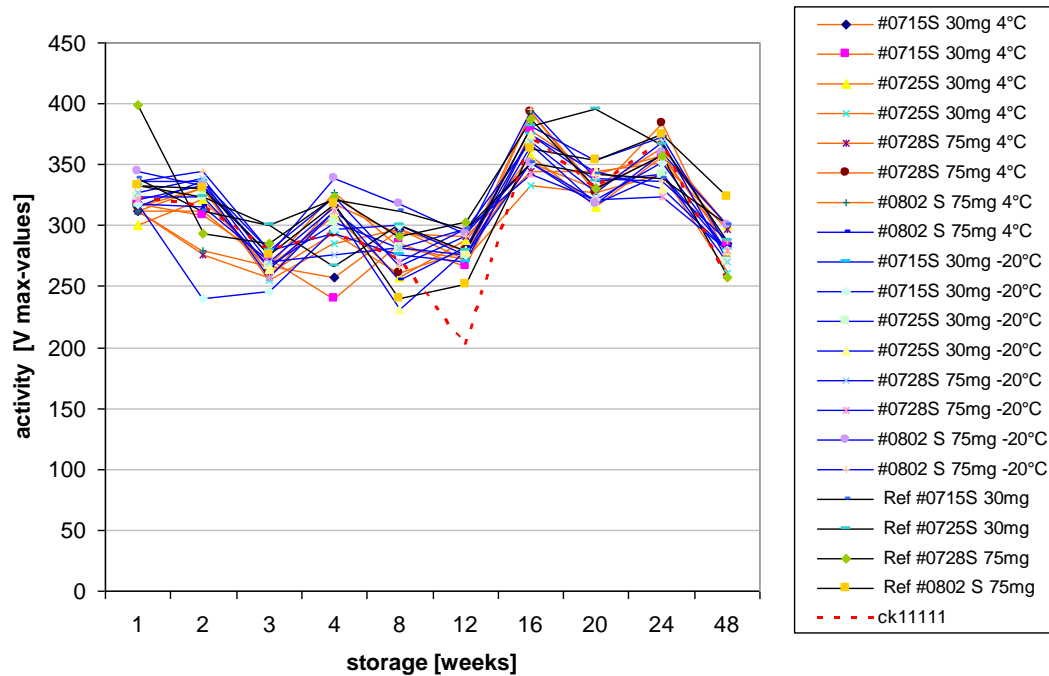
Stability data on NucleoMag VET

Stability of proteinase K (lyophilized)



Stability data on NucleoMag VET

Stability of proteinase K (reconstituted in buffer PB)



Stability data on NucleoMag VET

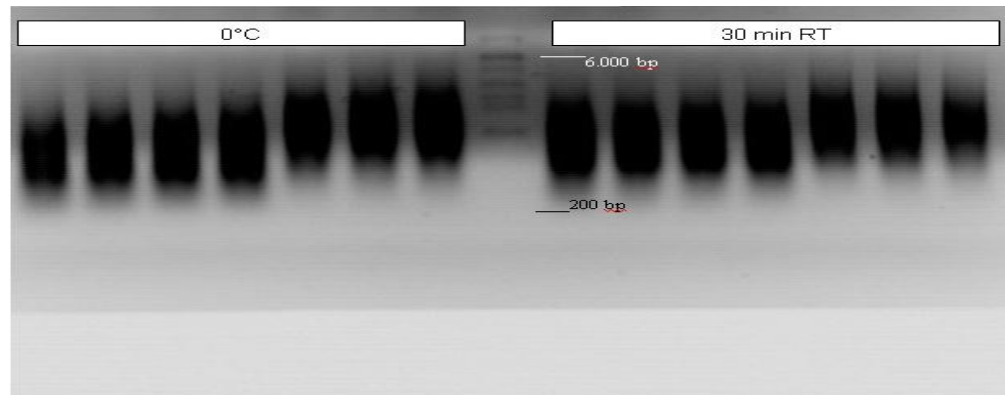
Stability carrier RNA (26 month)

Lots tested:

Lot C0809S, manufactured on December 3, 2008, supplier lot # 088K4022

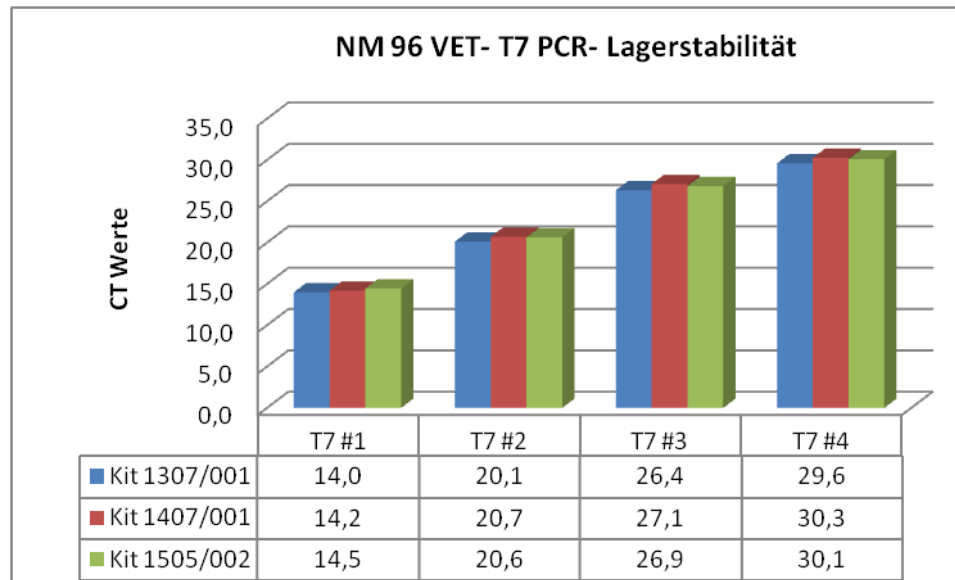
Lot C0609S, manufactured on October 10, 2006, supplier lot # 016K146

Lot	Value 1	Value 2	Mean value	Release criteria met
A260 (converted into µg/well)				
C0809S	7,25	7,46	7,35 µg/well	yes
C0609S	7,41	7,05	7,23 µg/well	yes
A260/280				
C0809S	3,42	3,41	3,38	yes
C0609S	3,40	3,38	3,39	yes
A260/228				
C0809S	3,78	3,68	3,71	yes
C0609S	3,73	3,73	3,73	yes



Stability data on NucleoMag VET

Stability of the kit (T7 DNA spike recovery, qPCR)



1407/001: 10 month

1305/001: 24 month

1307/001: new kit, reference