MACHEREY-NAGEL

NucleoMag® Pathogen

Automated purification of viral RNA / DNA and microbial DNA from clinical samples on the HAMILTON Genomic STARlet™ platform

Introduction

Isolation of pathogen nucleic acids (e.g. viral RNA and DNA, bacterial DNA) from clinical samples is the basis for a large variety of molecular tests that have become standard methodology in research and diagnostic laboratories.

Due to the diversity of clinical sample material – swabs, blood, plasma, body fluids, tissue biopsies, etc. – the isolation procedure itself often poses challenges to laboratory staff and workflows. The purification process needs to be suitable for a wide variety of sample materials. In addition, the molecular diagnostic market demands extraction methods that are adaptable on automation platforms, free of cross contaminations and reliable in terms of pathogen detection.

To meet these requirements MACHEREY-NAGEL developed the NucleoMag® Pathogen kit allowing the automated isolation of nucleic acids from various starting materials using magnetic bead technology.

MACHEREY-NAGEL and Hamilton were partnering to elaborate a protocol for automated magnetic bead based nucleic acid extraction expanding Hamilton's Genomic STARlet extraction capabilities by supplementing the workstation with MACHEREY-NAGEL's NucleoMag® SEP magnetic plate.

In this application note we demonstrate the utility and advantages of the automated NucleoMag® Pathogen extraction workflow from clinical samples on one of Hamilton's most popular workstations.

HAMILTON Genomic STARlet
Fully automated workflows to quickly isolate high-quality nucleic acids

Product at a glance

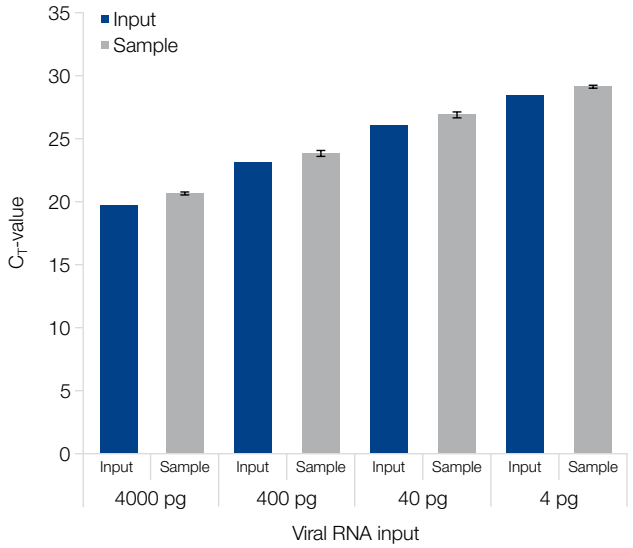
NucleoMag® Pathogen	
Technology	Magnetic beads
Sample material	< 200 µL whole blood, serum, plasma < 200 µL swab wash solution < 25 mg tissue < 200 µL feces
Target molecules	Viral RNA, viral DNA, microbial DNA from clinical samples
Fragments size	300 bp – approx. 50 kbp
Elution volume	50 – 200 µL

Genomic STARlet workstation	
Technology	Automated liquid handling platform optimized for nucleic acid purification workflows
Capacity	1–96 samples (≤ 200 µL sample volume)
Pipetting volume	1–1000 µL
Equipment/Features	CO-RE gripper, integrated heater shaker, 4 or 8 channel pipetting tool, sample auto load (optional), and many others

Material and Methods

The NucleoMag® Pathogen kit is designed for common clinical sample material, such as whole blood, swabs, serum or plasma, feces, and tissue. Up to 200 µL of liquid or homogenized sample material (e.g., swab wash solution) is mixed with Proteinase K, Carrier RNA (optional) and Lysis Buffer NPL1 prior to lysis incubation. The subsequent isolation is based on reversible adsorption of nucleic acids to paramagnetic beads (NucleoMag® B-Beads). Nucleic acid binding is enabled by addition of Binding buffer NPB2. After magnetic separation and removal of the supernatant, contaminants and salts are removed by three subsequent washing steps. The NucleoMag® B-Beads are air dried before highly pure nucleic acids are finally eluted under low ionic strength conditions in Elution Buffer NPE5. We demonstrate this automated purification workflow for spiked viral RNA and DNA exemplarily. The tailored protocol allows flexible processing of up to 96 samples per run.

Application data



High sensitivity detection of viral RNA recovered from liquid samples

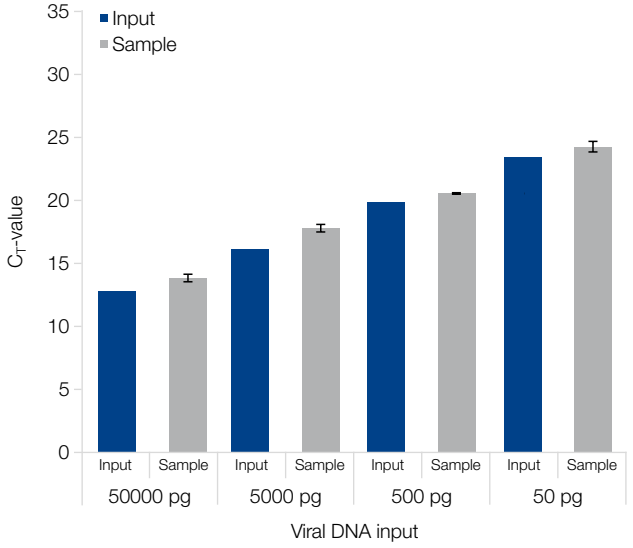
RNA was isolated from serum (200 µL; n = 3 for each dilution) using the NucleoMag® Pathogen kit on the Genomic STARlet workstation. MS2 bacteriophage RNA was spiked into the sample in a dilution series. RNA recovery and sensitivity of detection were evaluated by a subsequent Taqman® PCR assay for MS2 RNA using the SensiFast™ Probe One-Step Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. MS2 RNA purified from serum (sample) was detected with a sensitivity comparable to a control sample of the same RNA concentration (input) indicating an optimal recovery from the serum sample.

	A	B	C	D	E	F	G	H
1	27.85	*	26.11	*	26.29	*	26.49	*
2	*	25.82	*	26.16	*	25.87	*	27.05
3	27.03	*	26.22	*	26.33	*	25.93	*
4	*	26.53	*	26.27	*	26.06	*	26.22
5	26.57	*	26.35	*	25.99	*	25.91	*
6	*	26.70	*	26.27	*	26.28	*	26.96

■ Sample (C_T; positive control)
■ No sample (*C_T undetermined; negative control)

Cross contamination-free automated purification workflow

Positive (human DNA) and negative control (no DNA) samples (200 µL each) were arranged in a checkerboard board pattern on a 96-well deepwell plate and subjected to the NucleoMag® Pathogen procedure on the Genomic STARlet workstation. Presence of DNA in the eluates was examined by a qPCR assay for a human gDNA gene target. All positive samples were successfully amplified and detected (mean Ct = 26.39 ± 0.46). Absence of qPCR signal (CT undetermined) in the negative control samples indicates a cross contamination-free workflow.



High sensitivity detection of viral DNA recovered from liquid samples

DNA was isolated from human plasma (200 µL; n = 3 for each dilution) using the NucleoMag® Pathogen kit on the Genomic STARlet workstation. T7 bacteriophage DNA was spiked into the sample in a dilution series. DNA recovery and sensitivity of detection were evaluated by a subsequent Taqman® PCR assay for T7 DNA using the SensiFast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System. T7 DNA purified from human plasma (sample) was detected with a sensitivity comparable to a control sample of the same DNA concentration (input) indicating an optimal recovery from the plasma sample.

Automate your pathogen nucleic acid extraction from clinical samples

MACHEREY-NAGEL and Hamilton deliver a biologically verified solution for automated purification of pathogen nucleic acids, including viral RNA, viral DNA, and microbial DNA. We adapted the NucleoMag® Pathogen procedure to the fully automated Genomic STARlet system and demonstrate the suitability and utility for molecular testing workflows.

Your advantages at a glance

- Excellent recovery of nucleic acid from clinical samples
- High sensitivity of downstream qPCR assays
- Automated, cross contamination-free workflow
- Processing of NucleoSpin® and NucleoMag® kits on the same workstation

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® Pathogen	Magnetic bead-based kit for the isolation of viral RNA / DNA, and microbial DNA from clinical samples; including NucleoMag® B-Beads, buffers, Carrier RNA and Proteinase K	1 x 96 preps 4 x 96 preps	744210.1 744210.4
NucleoMag® SEP	Magnetic separator, for use with 96-well plates		744900
Genomic STARlet	Automated liquid handling platform with 4/8-channel pipetting channels, a CO-RE gripper, heater shaker, vacuum chamber, and many additional features		Hamilton*

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* For more detailed information, please visit www.hamiltoncompany.com/robotics. To find a Hamilton subsidiary or distributor in your area, please visit www.hamiltoncompany.com/contacts.