

Purification of viral nucleic acid from serum, plasma, cell-free biological fluids



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viral RNA:

**NucleoSpin® RNA Virus
NucleoSpin® RNA Virus F
NucleoSpin® 8 Virus
NucleoSpin® 96 Virus**

viral DNA:

**NucleoSpin® Blood
(NucleoSpin® RNA Virus, Virus F)
NucleoSpin® 8 Virus
NucleoSpin® 96 Virus**

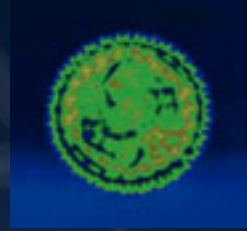
Purification of viral nucleic acid from tissue and cells

viral RNA:

NucleoSpin® RNA II / L / 96

viral DNA:

NucleoSpin® Tissue / 8 / 96

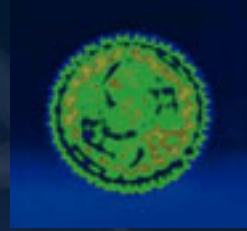


NucleoSpin[®] RNA Virus

the product family for the isolation of viral RNA
from serum and plasma

NucleoSpin[®] RNA Virus

RNA virus



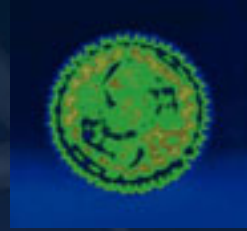
Features

150 µl of serum, plasma or any cell free biological fluids can be processed

up to 20 µg DNA or 50 µg RNA can be bound to the column with a recovery rate of 90%

the isolated nucleic acids can be used for PCR/RT-PCR reactions

NucleoSpin® RNA Virus



RNA virus

	NucleoSpin® RNA Virus	NucleoSpin® RNA Virus F	NucleoSpin® Virus	NucleoSpin® 96 Virus
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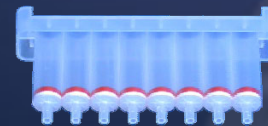
sample size:	150 µl	1000 µl	100 µl	100 µl
typ.analysis limit:	30 – 60 cp./ml	30 – 60 cp./ml	30 – 60 cp./ml	30 – 60 cp./ml
elution:	50 µl	50 µl	70 - 100 µl	70 -100 µl



mini



funnel



8-well



96-well

NucleoSpin[®] RNA Virus

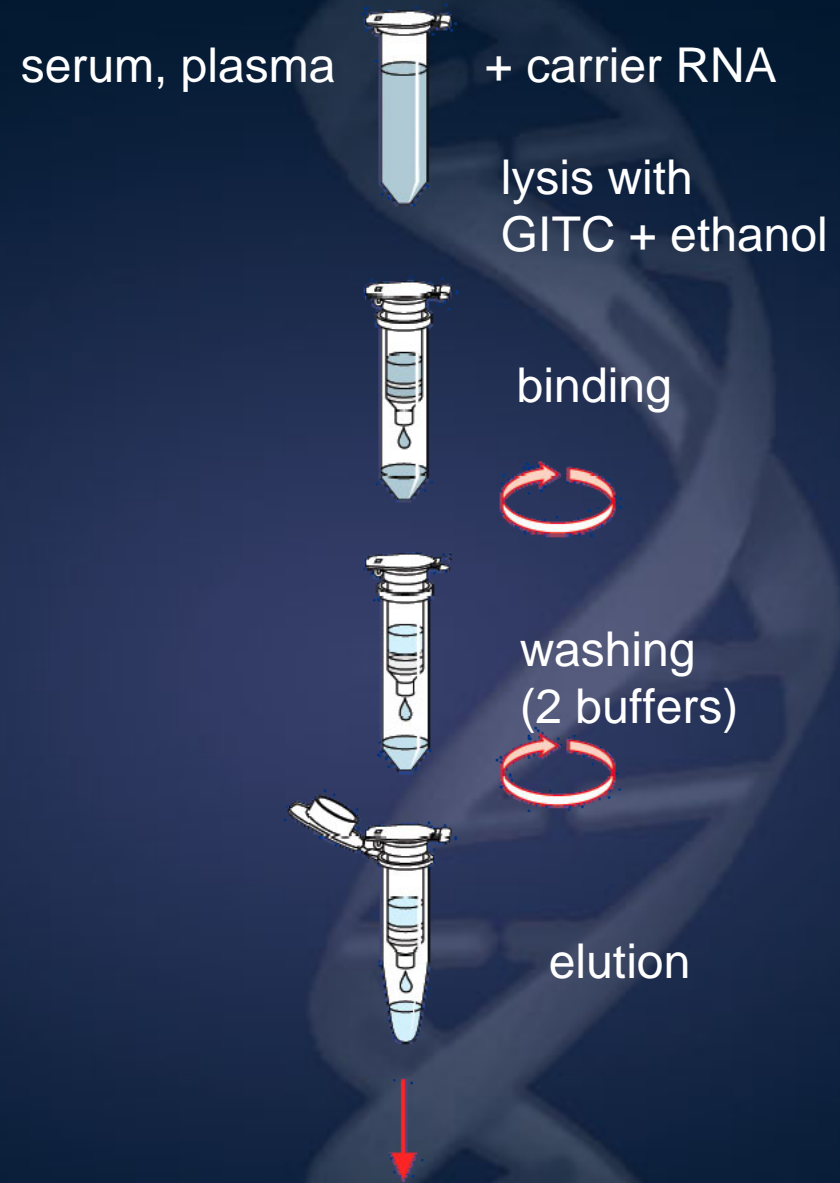
Procedure

Lysis: lysis buffer RAV1 + carrier RNA
(+ Proteinase K not incl.)

Binding: add ethanol
water shell of the DNA is destroyed by chaotropic salt – silica membrane serves as binding partner for the DNA

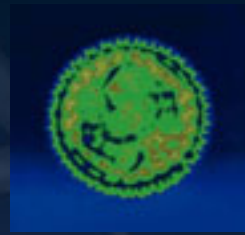
Washing: 1 x buffer RAW (high salt)
2 x buffer RAV3 (low salt)

Elution: for RNA use Rnase free water
for DNA use buffer RE
water shell of the DNA recovers – DNA is released from the silica membrane



NucleoSpin® RNA Virus

RNA virus



Actually tested

HIV

HCV

CMV

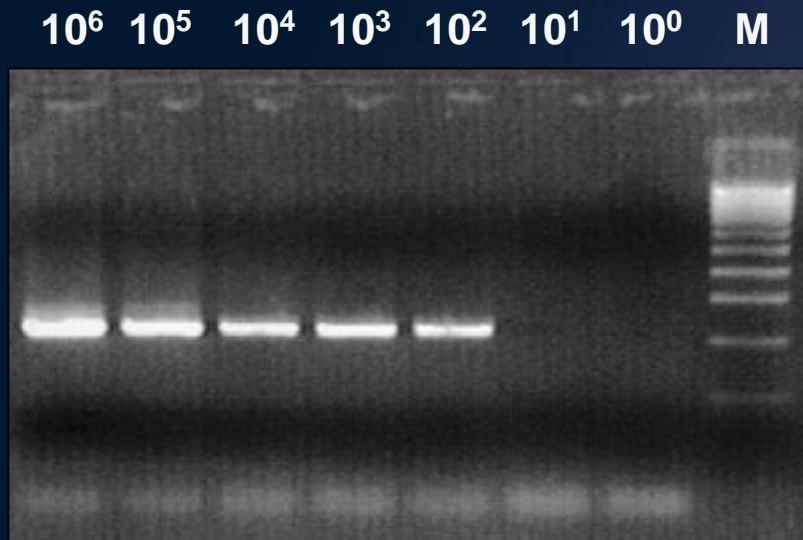
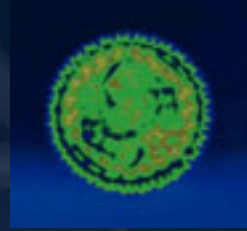
Parvovirus B19

Mycoplasma (cell wall-free microorganism)



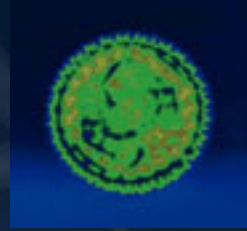
NucleoSpin[®] RNA Virus

Original data

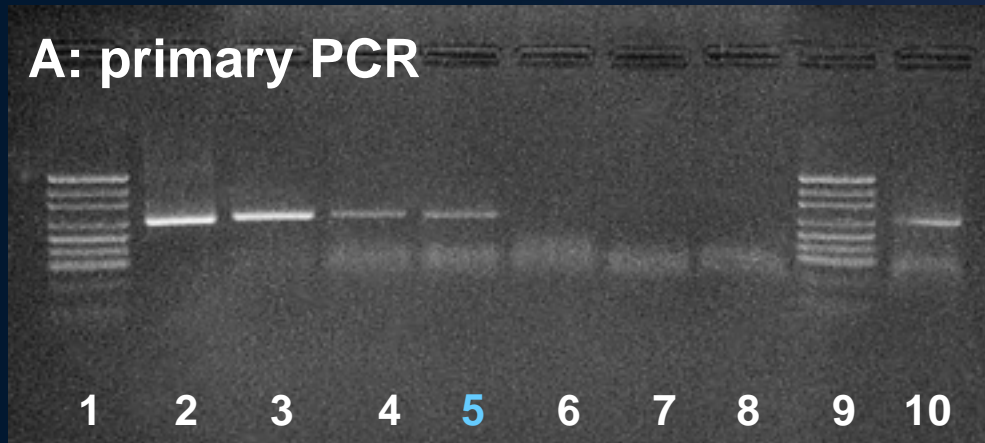


RT-PCR of HIV RNA
from supernatant of H9 cell culture
in logarithmic dilution steps

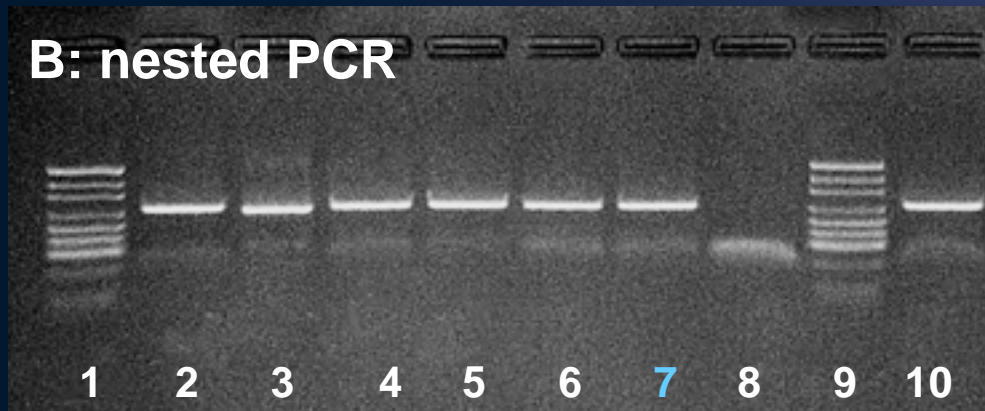
NucleoSpin® RNA Virus



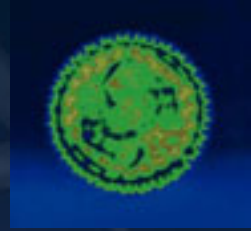
Original data (HCV)



1: marker
2: HCV - 50 000 cp/ml
3: HCV - 5 000 cp/ml
4: HCV - 2 500 cp/ml
5: HCV - 1 250 cp/ml
6: HCV - 500 cp/ml
7: HCV - 250 cp/ml
8: negative control
9: marker
10: positive control



1: marker
2: HCV - 5 000 cp/ml
3: HCV - 500 cp/ml
4: HCV - 250 cp/ml
5: HCV - 125 cp/ml
6: HCV - 62.5 cp/ml
7: HCV - 31.25 cp/ml
8: negative control
9: marker
10: positive control

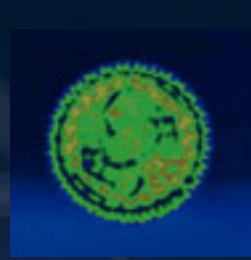


NucleoSpin[®] RNA Virus F

MACHEREY-NAGEL



NucleoSpin® RNA Virus F



Features

**Handling of larger volume :
up to 1 ml with one loading step**

**Elution volume is still 100 µl – highly
concentrated sample**

**High sensitivity: 20 - 50 copies/ml of RNA
virus can be detected**

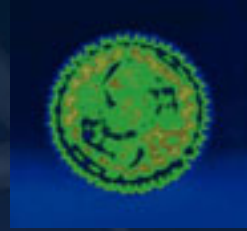
Closed system: No cross contamination

Collection and elution tubes are included

Patent pending

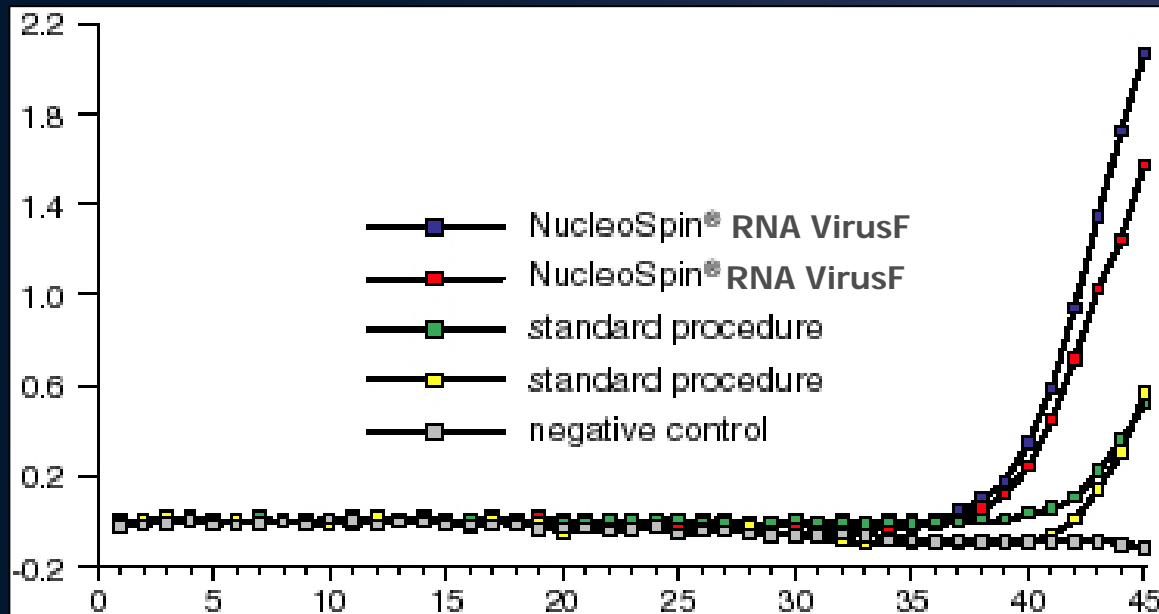


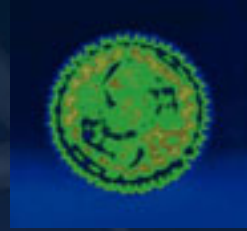
NucleoSpin® RNA Virus F



Original data

TAQ MAN analysis of HCV from a cell supernatant





NucleoSpin[®] 8 Virus
NucleoSpin[®] 96 Virus

NucleoSpin® 8/96 Virus

Features

**Simultaneous extraction of viral RNA and DNA
from 100 µl serum, plasma, cell-free fluids**

Proteinase K included

HCV, HBV, HAV, HIV, HSV, HPV, VZV, EBV, parvovirus B19

**Isolated nucleic acids can be used for RT-PCR,
PCR, and other subsequent reactions**

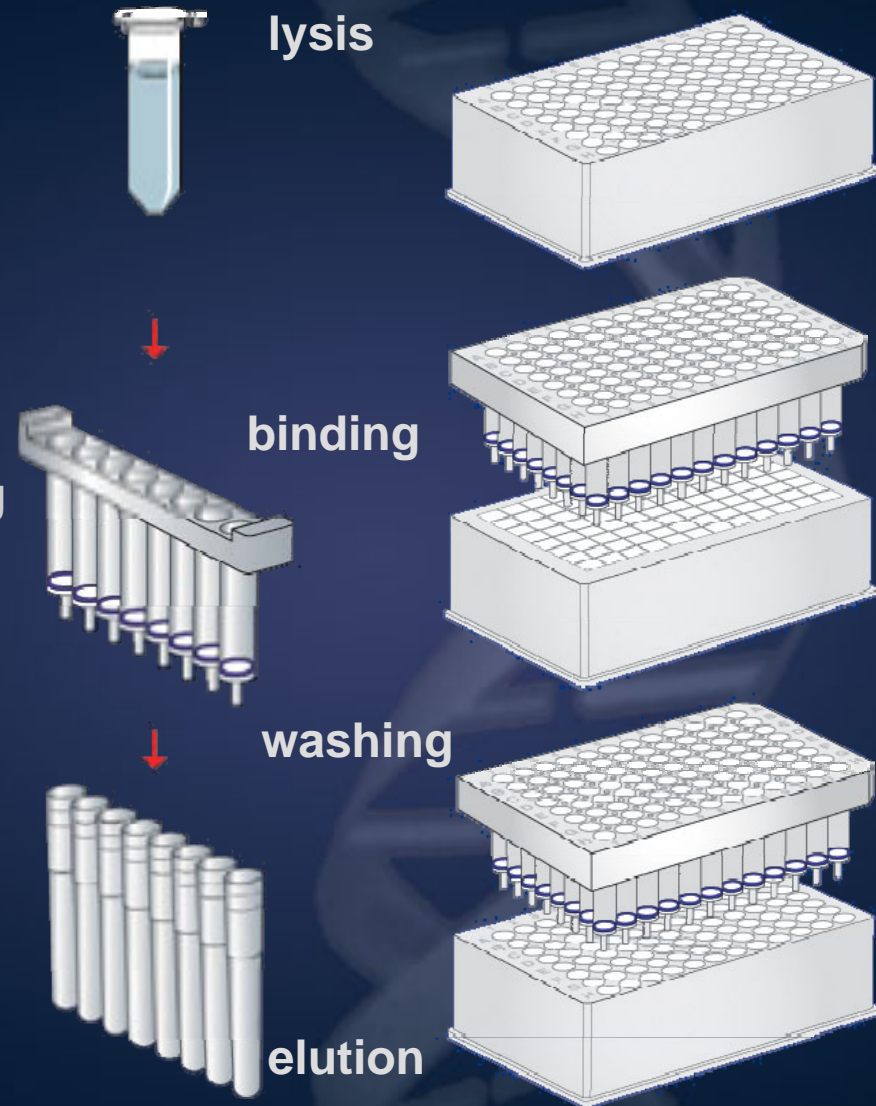
NucleoSpin® 8/96 Virus

Lysis: lysis buffer RAV1 + carrier RNA
(+ Proteinase K not incl.)

Binding: add ethanol
water shell of the DNA is
destroyed by chaotropic salt –
silica membrane serves as binding
partner for the DNA

Washing: 1 x buffer RAW (high salt)
2 x buffer RAV3 (low salt)

Elution: for RNA use Rnase free water
for DNA use buffer RE
water shell of the DNA recovers –
DNA is released from the silica
membrane



NucleoSpin® Multi-96 Virus

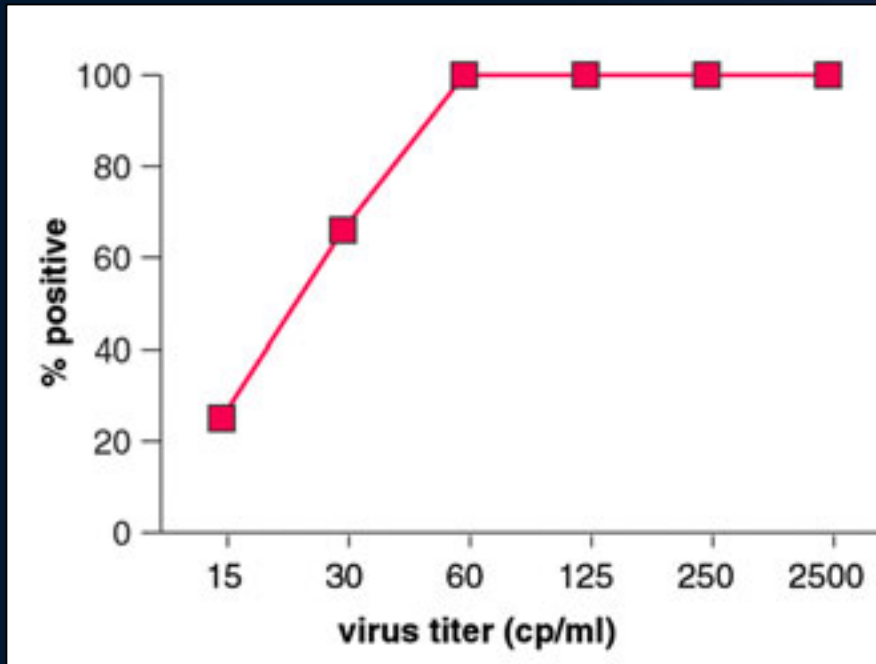
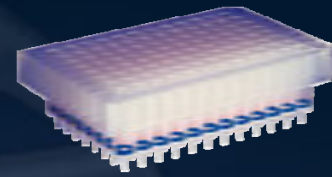
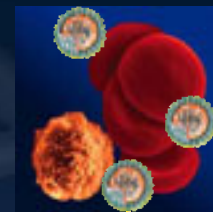


Fig. 5.3 Highly Sensitive Detection of Viral RNA

HCV RNA was purified from 96 plasma samples (100 μ l). A dilution series was processed using NucleoSpin® Multi-96 Virus (12 samples each).

Percentage of positive RT-PCR samples (COBAS Amplicore, Roche Diagnostics) at low virus titers are shown.

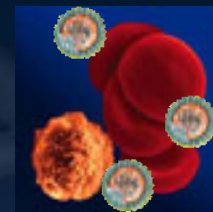


NucleoSpin[®] Blood

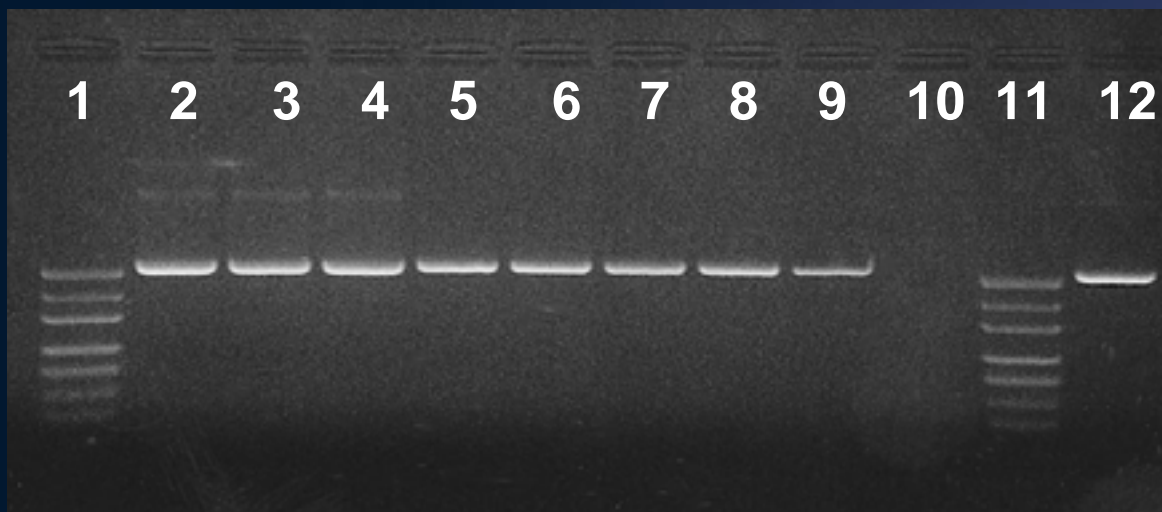
DNA virus from body fluids

NucleoSpin® Blood

DNA virus



NucleoSpin® Blood - viral DNA (HBV)



- 1: marker
- 2: HBV - 500 000 cp/ml (undiluted sample)
- 3: HBV - 250 000 cp/ml
- 4: HBV - 100 000 cp/ml
- 5: HBV - 50 000 cp/ml
- 6: HBV - 25 000 cp/ml
- 7: HBV - 5 000 cp/ml
- 8: HBV - 500 cp/ml
- 9: HBV - 100 cp/ml
- 10: negative control
- 11: marker
- 12: positive control



MACHEREY-NAGEL

