Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information - ESI

Automated nucleic acid extraction from whole blood, *B . subtilis*, *E. coli*, and *Rift Valley fever* virus on a centrifugal microfluidic LabDisk

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The supporting material includes:

1) Spin protocol for rotation of centrifugal microfluidic cartridge

Table S1: Predefined spin protocol used for automated nucleic acid extraction on LabDisks.

Frequency [Hz]	Duration [s]	Notes
10	1	Sample and reagents (except binding buffer) are gated radially outwards
6 <--> 10	900	Rehydration of prestored bead pellet
2.5	40	Priming of capillary siphon
0	60	Priming of capillary siphon
17	40	Transport of binding buffer into extraction chamber
3.5 <--> 10	60	Binding of released nucleic acids to beads
Beadtransport	80	Transport of beads in washing buffer 1
3 <--> 10	60	Mixing of beads in washing buffer 1
Beadtransport	80	Transport into washing buffer 2
3 <--> 10	60	Mixing of beads in washing buffer 2
Beadtransport	300	Transport into elution buffer
4 <--> 10	120	Elution of DNA from bead surface

2) Magnet Set-up

Magnet set-up for transport of magnetic beads between different microfluidic chambers (**Fig S1**). Transport magnet and collection magnet are mounted to a magnet holder at radial distances of r_1 = 42 mm and r_2 = 52.5 mm. Distance between transport magnet and LabDisk extraction chamber is about 0.5 mm. Transport and collection magnet have been stacked from standard NdFeB permanent magnets (transport magnet: #S-07-05-N, #S-05-08-N, #S-04-03-N und #S-02-02-N; collection magnet: #S-07-05-N, #S-06-06-N and #S-06-03-N) purchased from supermagnete.de, Germany.

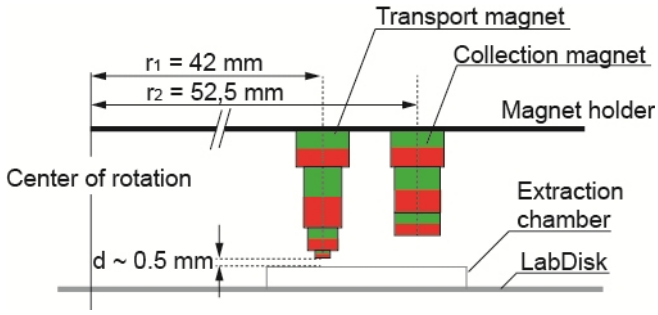


Fig S1: Cross section of magnet set-up (transport and collection magnet) used for transport of magnetic beads between different microfluidic chambers.

3) Processing of LabDisk cartridges

LabDisk cartridges were processed in a small, light-weight and portable processing device developed by QIAGEN Lake Constance GmbH, suitable for perspective application at the point-of-care **Fig. S2**. As a technical novelty, we included a second magnet “collection magnet”, for collection of magnetic beads within the liquid phase. After collection, the “transport magnet” pulls the beads out of the liquid phase. With

the improved magnet set-up, magnetic beads could be transported between liquid reservoirs with up to 1 mL volume thereby allowing to process sample volumes similar to standard bench top nucleic acid extraction protocols.

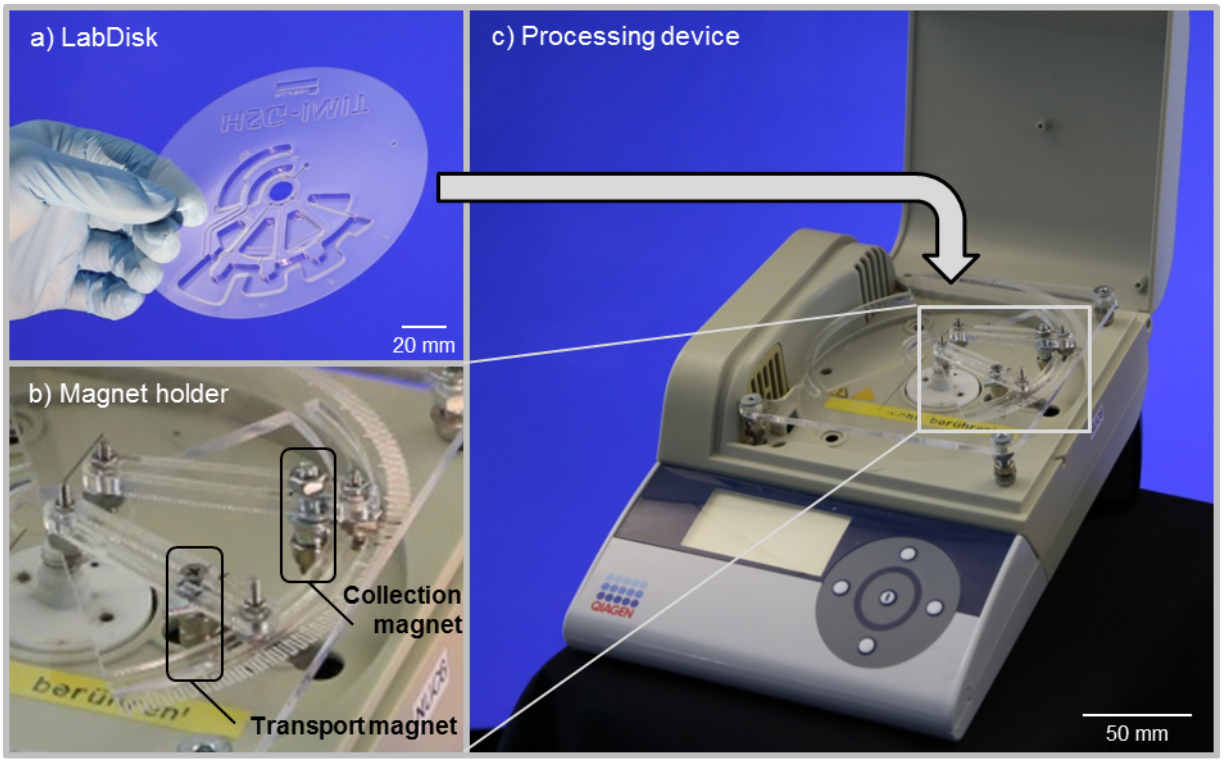


Fig. S2: Set-up for nucleic acid extraction experiments. (a) LabDisk cartridge for automated nucleic acid extraction. The LabDisk is placed in the processing device. (b) Magnet holder with two stacks of magnets for collection and transportation of magnetic beads is mounted above the disk. (please see also *ESI)* (c) Prototype processing device, developed by QIAGEN Lake Constance GmbH (Germany) in collaboration with HSG-IMIT. Its dimensions are 25 x 17,5 x 8,5 cm³ and its weight is less than 2 kg. Via connection to a laptop, it is possible to run multistep spin protocols with controlled spin frequency (up to 90 Hz), acceleration and deceleration (0.1 – 50 Hz s^{-1}) as well as defined positioning with an accuracy of 0.1°.

4) Implemented fluidic functionality

For transporting magnetic beads between different, liquid filled microfluidic chambers, the LabDisk cartridge is slowly rotated from a predefined first to a second azimuthal position while the collection magnet collects the beads within the lysate **Fig. S3 a**. The LabDisk is rotated to a third azimuthal position allowing the transport magnet to pull the collected magnetic beads out of the lysate **Fig. S3 b.** The LabDisk is moved to a fourth position while the magnetic beads follow the transport magnet to an azimuthal position radially inwards of the washing buffer **Fig. S3 c.** After acceleration to 10 Hz, the magnetic beads are centrifuged into the washing buffer **Fig. S3 d**. Steps a – d are repeated to transport the magnetic beads into the chamber with the second washing-buffer and finally into the chamber containing the elution-buffer, where the purified nucleic acids are released from the surface of the beads. Photographs of the bead transport sequence are depicted in **Fig. S3 A – D**. A video of the bead transport is available as *electronic supplement*.

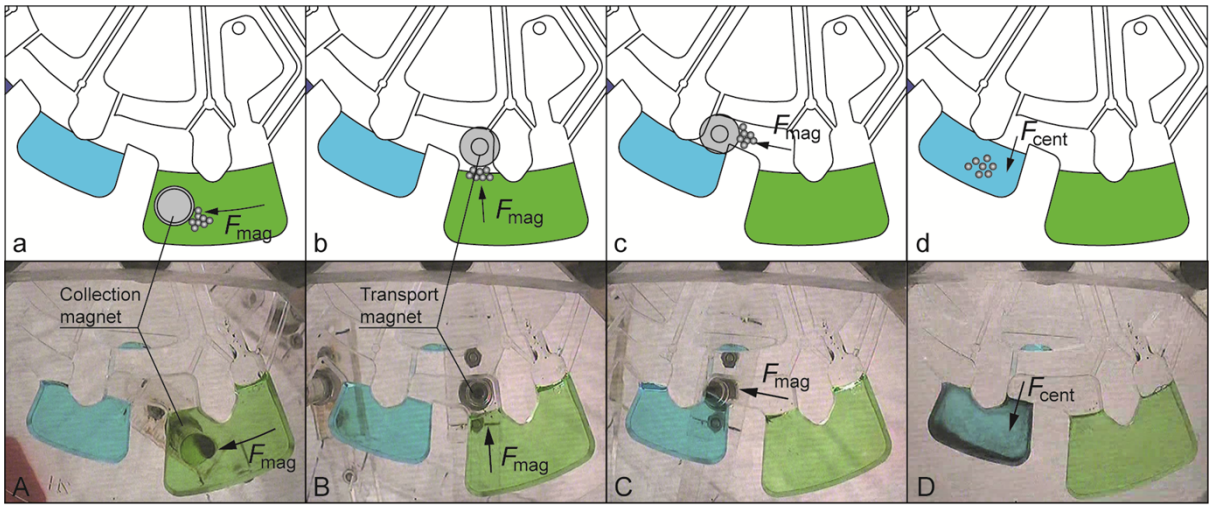


Fig. S3: Step-by-step bead transport between two microfluidic chambers. Depending on the azimuthal positioning of the disk with respect to collection and transport magnet, beads are collected within the liquid (a), pulled out of the liquid (b) and transported through an air gap that separates adjacent chambers (c). Finally, the beads are centrifuged radially outwards into a second liquid. (d)

5) Pelleting of magnetic beads

For fabrication of magnetic bead pellets, 20 µL droplets of vortexed magnetic bead suspension (MAG Suspension M, Analytik Jena, Germany) were dispensed on the hydrophobic surface of balance dish and left for air drying. After drying, bead pellets were placed in the LabDisk.

6) DNA extraction from whole blood via QIAGEN spin columns

The following protocol (depicted in **Table S2**) was used for extraction of DNA from whole blood using QIAGEN spin columns.

Table S2: Manufacturers protocol for DNA extraction using QIAGEN spin columns.

Step No.	Action
1	Mix 200 whole blood, 20 µL protease and 200 µL lysis buffer AL in 1.5 mL Tube
2	Vortex (15 seconds) and incubate (10 min)
3	Add 200 µL ethanol for DNA binding
4	Vortex (15 seconds)
5	Transfer mixture to spin column; Place spin column in centrifuge
6	Centrifuge 1 min / 8000 rpm
7	Exchange collecting vessel
8	Add 500 µL washing buffer AW 1 to spin colum
9	Centrifuge 1 min / 8000 rpm
10	Exchange collecting vessel
11	Add 500 µL washing buffer AW 2 to spin column
12	Centrifuge 3 min / 13200 rpm
13	Exchange collecting vessel against 1.5 mL Safe-Lock tube
14	Add 100 µL RNAse free water for elution
15	Incubate 1 min / 25 °C
16	Centrifuge 1 min / 8000 rpm; Storage of eluate at -20°C

7) Composition of PCR mixture

Composition of the PCR mixtures for real-time PCR and real-time reverse transcriptase-PCR based quantification of recovered DNA and RNA, respectively, is depicted in **Table S3.**

Table S3: Composition of PCR and RT-PCR reaction mixes for real-time PCR based amplification of extracted DNA and RNA

		B. subtilis / *E. coli*	Human blood	*Rift valley fever*
	Conc. [µM]	Vol [µL]	Vol [µL]	Vol [µL]
Template		1	1	1
F. primer	10	0.3	0.3	0.4
R. Primer	10	0.3	0.3	0.4
Probe	10	0.2	0.2	0.1
Water		3.2	6.8	3
PCR Mix		5 [1)]	1.4 [2)]	5 [3)]
RT Mix		---	---	0.1

F. primer = Forward primer; R. primer = Reverse Primer;
[1] QuantiTect Probe PCR Master Mix (QIAGEN, Germany)
[2] composed of 0.2 µL DNA Polymerase, 0.2 µL dNTP, 1 µL PCR buffer HotStarTaq Plus (QIAGEN, Germany);
[3] RT QuantiTect PCR Master Mix (QIAGEN, Germany)

8) Sequences of used primers and probes

Table S4: Sequences of used primers and probes for real-time PCR and real-time reverse transcriptase PCR based amplification of extracted nucleic acids.

Whole blood (Human growth hormone gene)	
Forward primer	GGGAGAGGCAGCGACCTGTA
Reverse primer	GGAGAGCAAGAGGCCAGCAC
Probe	FAM-TGGGACGGGGGCACTAACCC-BHQ1
B. subtilis (sspE gene)	
Forward primer	GCTAGCGAAACAAACGCTCAGCAA
Reverse primer	ACTTCCACCCGAAGATGAAGTGCT
Probe	FAM-AGCTGGACAACAAGGTCAATTCGGCA-BHQ1
E. coli (pal gene)	
Forward primer	GGCAATTGCGGCATGTTCTTCC
Reverse primer	TGTTGCATTTGCAGACGAGCCT
Probe	FAM-ATGCGAACGGCGGCAACGGCAACATGT-BHQ1
Rift Valley fever	
Forward primer	TGCCACGAGTYAGAGCCA
Reverse primer	TTGAACAGTGGGTCCGAGA
Probe	FAM-TCCTTCTCCCAGTCAGCCCCAC-BHQ

FAM: Fluorescein; BHQ1: Blackhole quencher 1

9) Recovery of DNA and RNA after extraction

Recovery of *B. Subtilis* DNA from LabDisk based extraction and manual reference extraction with respect to inserted colony forming units.

Inserted number of *B. subtilis* colony forming units	LabDisk recovered number of DNA copies (mean ± std. dev)	Reference recovered number of DNA copies (mean ± std. dev)	Ratio LabDisk / Manual Reference
1.1×10^6	$(2.4 \pm 1.6) \times 10^6$	$(4.1 \pm 1.2) \times 10^6$	58.2 %
1.1×10^5	$(2.3 \pm 0.9) \times 10^5$	$(3.7 \pm 1.1) \times 10^5$	63.2 %
1.1×10^4	$(3.2 \pm 2.4) \times 10^4$	$(3.8 \pm 2.1) \times 10^4$	84.0 %
1.1×10^3	$(4.2 \pm 5.5) \times 10^3$	$(4.3 \pm 3.2) \times 10^3$	98.5 %
5.3×10^2	2.6×10^3 (1.8×10^3; 6.8×10^3) [a]	2.6×10^3 (1.8×10^3; 6.8×10^3) [a]	---
1.1×10^2	1.5×10^3 (0.9×10^3; 3.3×10^3) [a]	6.0×10^2 (0; 1.2×10^3) [a]	---
5.3×10^1	1.7×10^3 (1.1×10^3; 4.0×10^3) [a]	No amplification	---

[a] Error is depicted as 95 % confidence interval between (min; max)

Recovery of *E. coli* DNA from LabDisk based extraction and manual reference extraction with respect to inserted colony forming units.

Inserted number of *E. coli* colony forming units	LabDisk recovered number of DNA copies (mean ± std. dev)	Reference recovered number of DNA copies (mean ± std. dev)	Ratio LabDisk / Manual Reference
1.5×10^6	$(1.3 \pm 0.2) \times 10^6$	$(1.7 \pm 0.6) \times 10^6$	76.5 %
1.5×10^5	$(2.4 \pm 0.9) \times 10^5$	$(2.9 \pm 0.8) \times 10^5$	81.9 %
1.5×10^4	$(3.4 \pm 1.2) \times 10^4$	$(3.3 \pm 1.7) \times 10^4$	102.1 %
1.5×10^3	$(2.1 \pm 1.4) \times 10^3$	$(4.6 \pm 3.9) \times 10^3$	45.3 %
1.5×10^2	1.0×10^3 (0.5×10^3; 2.1×10^3) [a]	1.3×10^3 (0.8×10^3; 2.8×10^3) [a]	---

[a] Error is depicted as 95 % confidence interval between (min; max)

Yield of *Rift valley fever* virus RNA from LabDisk based extraction and manual reference extraction with respect to inserted genome equivalents.

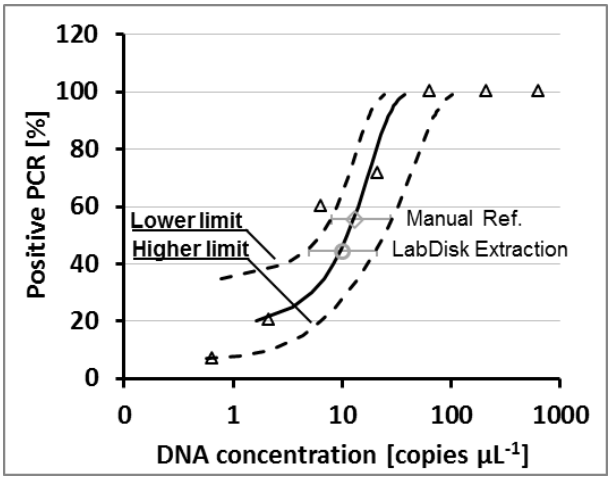
Inserted number of *Rift Valley Fever virus* genome equiv.	LabDisk recovered number of RNA copies (mean ± std. dev)	Reference recovered number of RNA copies (mean ± std. dev)	Ratio LabDisk / Manual Reference
6.6×10^4	$(8.2 \pm 6.2) \times 10^4$	$(24.4 \pm 13) \times 10^4$	34.2 %
6.6×10^3	$(3.3 \pm 4.1) \times 10^3$	$(11.2 \pm 8.1) \times 10^3$	29.5 %
6.6×10^2	1.0×10^2 (0; 3.2×10^2)	6.5×10^2 (4.5×10^2; 9.7×10^2)	---

10) PROBIT Analysis for quantification of DNA / RNA recovery

PROBIT regression analysis can be used for estimation of DNA and RNA copy numbers at low concentrations. Replicate PCR testing at these dilutions is dominated by statistical effects and thus results in a number of positive and negative PCR amplifications. The ratio of positive amplifications from the overall number of PCR replicates can then be compared to ratios derived from replicate PCR testing of a reference dilution series as depicted below. Regression analysis is supported by the statistical software SPSS. [1, 2, 3]

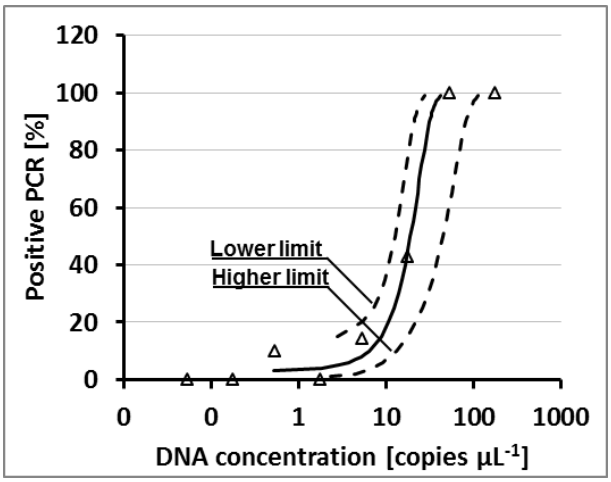
Data for PROBIT analysis of *E. coli*. Left: Results of replicate PCR testing of a dilution series with known DNA copy number and corresponding probability for positive amplification (Ratio pos / PCR replicates). Right: PROBIT plot. Probability of positive PCR (solid line = mean value; dashed lines 95 % confidence) as a function of the DNA concentration derived from replicate PCR testing at defined DNA concentrations (triangle). Ratio of positive PCR's to overall number of replicates of unknown samples is used for quantification. *E. coli* manual reference (diamond) and LabDisk extraction (circle) for an inserted number of 1.5 x 10^2 CFU has been depicted

DNA Copies	PCR Replicates	Positives	Ratio Pos. / PCR Replic. [%]
2110	5	5	100
634	5	5	100
211	7	7	100
63.4	7	7	100
21.1	7	5	71.4
6.34	10	6	60
2.11	10	2	20
0.634	15	1	6.7



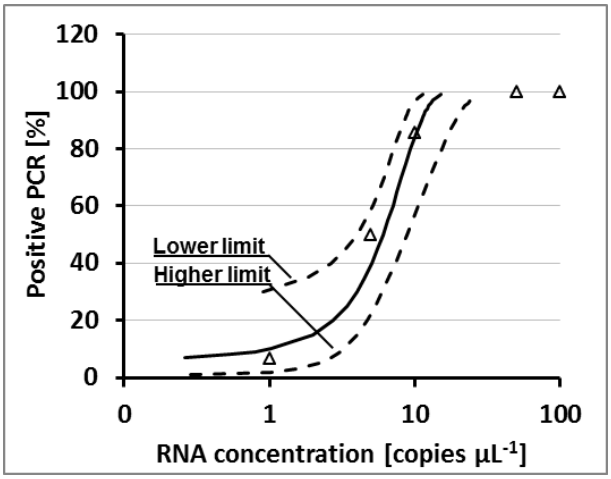
Data for PROBIT analysis of *B. subtilis*.

DNA Copies	PCR Replicates	Positives	Ratio Pos. / PCR Replic. [%]
176	5	5	100
52.9	5	5	100
17.6	7	3	42.9
5.29	7	1	14.3
1.76	7	0	0
0.529	10	1	10
0.176	10	0	0
0.0529	15	0	0



Data for PROBIT analysis of *Rift valley fever virus*

RNA Copies	PCR Replicates	Positives	Ratio Pos. / PCR Replic. [%]
100	3	3	100
50	7	7	100
10	7	6	85.7
5	10	5	50
1	15	1	6.7



[1] M. Smieja et al., *J. Clin. Microbiol*., **2001**, 39,1796–1801.

[2] M. Weidmann et al.,, *J. Clin. Virol*., **2010**, 48, 187–192.

[3] O. Strohmeier et al., *Lab Chip*, **2013**, 13, 146–155