979658

National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

RIVM
Centre for Infectious Diseases
A. van Leeuwenhoeklaan 9
3721 MA Bilthoven
Postbus 1
3720 BA Bilthoven
www.rivm.nl

T 5.1.2e
info@rivm.nl

Evaluation of different processing cartridges for total nucleic acid extraction using the Roche MagNA Pure96 system

Evaluation Report
Final

Authors:
5.1.2e
5.1.2e

Copyright notice and reservation of rights
All copyrights in the content of this report, including any appendix (s), (including texts and images) are expressly reserved. This report is confidential and intended only for the addressee. Provision to and use by others than the addressee is not permitted without the consent of RIVM. Likewise, any other form of disclosure, reproduction, distribution and / or provision of the content of this report is not permitted, unless you are explicitly obliged to do so by law, have given explicit permission to RIVM or have clearly made the relevant information public itself. Manipulation of this report is never permitted.

Introduction

The official test materials such as processing cartridges for total nucleic acid (TNA)-extraction used in the Roche MagNA Pure96 system to support COVID-19 diagnostics are produced abroad by Roche. Due to the uncertainty of delivery by Roche through the regular channels alternative processing cartridges are produced. In this evaluation both Bioplastics-cartridges (Bioplastics, Landgraaf) as well as Helvoet-cartridges (Helvoet, Tilburg) are evaluated to see if they perform similar to the Roche-processing cartridges.

Methods to test and acceptance criteria

Main parameters to test are fit, leakage, contamination, sensitivity, inhibition and accuracy. To be accepted, the processing cartridges produced by Bioplastics and by Helvoet should fulfil the following criteria compared with processing cartridges produced by Roche.

- Fit: is tested during a MagNA Pure96 extraction-run. The cartridges should fit on-board, and during the extraction-protocol the needles and tips should not be obstructed while moving according to the x- y- and z-programmed coordinates described in the extraction-protocol.
- Leakage: is tested during and apart from a MagNA Pure96 extraction-run. During-run test: after the run is finished there should not be any fluid underneath the cartridges. Apart-from-run test: the cartridges are filled with water and incubated for one hour on top of a tissue. After one hour the tissue should be dry by visual check.
- Contamination: to check for dripping of fluid during the extraction process, for instance due to static charge originating from movement of tips along the processing cartridges, the processing cartridges are filled with virus-containing samples surrounded by negative control samples. The adjacent negative control samples should not give positive results in the virus-target-qPCR. In addition, after the run no visible leakage from the tips should be noticed on top of the cartridges and on the deck, and the drop-catcher should not show any sign of dripping.
- Sensitivity: to test if TNA extracted from the clinical sample binds to the plastic of the cartridges, five virus positive samples are extracted in a 10-fold dilution series (clinical sample is diluted in MEM-medium). These five samples should give similar dilution in the series positive and similar Cp value results in qPCR after TNA-extraction in both Bioplastics-cartridges and Helvoet-cartridges compared to the gold standard Roche-cartridges. The difference in Cp-value between both Helvoet- and Bioplastics extraction-runs compared to the Roche extraction-run for each individual sample and dilution should not be more than 1 Cp-value.
- Inhibition: to test if any chemical reaction of the plastic with the reagents used in the extraction process or any inhibitory residues released from the plastic will result in inhibition during the qPCR-process, the clinical samples used during this validation are run with an internal-control equine arteritis virus (EAV) spike. All eluates should give a similar Cp-value in the internal-control qPCR by cartridge type. A Cp-value difference of <1 Cp-value compared with the median of all measured EAV-Cp-values is tolerated. Between cartridge types the EAV-Cp-values should not be statistically significant different (t-test p value > 0.05)
- Accuracy: all found Cp-values of all clinical samples and dilution series are compared. The difference between the three extraction-runs for each individual sample and target using the three types of processing cartridges should not be more than 1 Cp-value.

Material & Methods

Fit:

Nine processing cartridges produced by Helvoet and nine processing cartridges produced by Bioplastics are tested to fit on board of the MagNA Pure96 system. Three processing cartridges of each type are used during an extraction run. The remaining six are only tested to fit in the drawers of the system.

Leakage:

Three processing cartridges of each type are used during an extraction run. After the run is finished, the drawers are checked to see if the surface contains fluid.

Six processing cartridges produced by Bioplastics and Helvoet are filled with water and placed on a tissue. After one hour the tissue is checked for leakage.

Contamination:

Eight clinical samples were selected (Table 1) that are confirmed virus positive, tested in the appropriate qPCR and diluted 10-fold to obtain a viral load measured in qPCR with a Cp-value between 17 and 24. Five fecal samples positive for norovirus, adenovirus or rotavirus and three throat swabs in GLY-transport medium positive for SARS-CoV-2 were selected. From each fecal sample a small amount of feces collected with an inoculation loop was resuspended in 1 ml MEM-medium and centrifuged for 5 minutes at 10000 rcf. Six hundred µl of the supernatant was drawn and mixed with 825 µl MagNA Pure lysis buffer with standard amount of EAV included (internal control spike) and yeast-tRNA. Each throat swab in transport medium was vortexed and 600 µl of the medium was drawn and mixed with 825 µl MagNA Pure lysis buffer with standard amount of EAV included (internal control spike) and yeast-tRNA. From all samples three aliquots of 450 µl were pipetted into the three types of processing cartridges produced by Bioplastics, Helvoet or Roche in a way that all samples were surrounded by negative control samples (Figure 1). These negative samples contained 200 µl GLY-transport medium and 275 µl MagNA Pure lysis buffer with standard amount of EAV included (internal control spike) and yeast-tRNA.

***Table 1**. Overview of clinical samples selected for extraction with MagNA Pure 96 system*

Sample Name	Target	Clinical sample
1	Norovirus type 1 (NV1)	feces
2	Norovirus type 1 (NV1)	feces
3	Norovirus type 2 (NV2)	feces
4	Adenovirus (AdV)	feces
5	Rotavirus (RV)	feces
6	SARS-CoV-2 virus (CoV)	throat swab in GLY transport medium
7	SARS-CoV-2 virus (CoV)	throat swab in GLY transport medium
8	SARS-CoV-2 virus (CoV)	throat swab in GLY transport medium

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC	NC	NC	NC	NC	NC	Virus undiluted				
B	NC	Sample 1 NV1	NC	Sample 7 CoV	NC	Sample 5 RV	NC	Virus 10-1				
C	NC	NC	NC	NC	NC	NC	NC	Virus 10-2				
D	NC	NC	NC	NC	NC	NC	NC	Virus 10-3				
E	NC	Sample 3 NV2	NC	Sample 4 AdV	NC	Sample 8 CoV	NC	Virus 10-4				
F	NC	NC	NC	NC	NC	NC	NC	Virus 10-5				
G	NC	NC	Sample 6 CoV	NC	Sample 2 NV1	NC	NC	Virus 10-6				
H	NC	NC	NC	NC	NC	NC	NC	Virus 10-7				

***Figure 1.** Positioning of the positive samples surrounded by negative control samples (NC) in the processing cartridge, and positioning of the 10-fold dilution series of combined viral targets in column "8"*

Sensitivity:

To prepare a 10-fold dilution series, 100 µl of each sample (Table 1) was combined to obtain a total of 800 µl combined virus sample. Six hundred µl of the combined sample was drawn and mixed with 825 µl MagNA Pure lysis buffer with EAV included (internal control spike); 'Virus undiluted' in Figure 1 column '8'. From the 200 µl remainder combined virus sample 60 µl was drawn to prepare a 10-fold dilution in 540 µl GLY-transport medium and mixed with 825 MagNA Pure lysis buffer, etcetera; Virus 10-1 – 10-7 in Figure 1 column '8'. From

this dilution series three aliquots of 450 µl were pipetted into the three types of processing cartridges (Figure 1; column '8').

Inhibition:

All the samples in table 1 and negative control samples were spiked with the same amount of EAV used as a standard internal control. The standard amount of EAV that is added to the MagNA Pure lysis buffer provides a Cp value between 25.9 and 27.2

Contamination, Sensitivity, Inhibition, Accuracy:

All samples were extracted on a MagNA Pure 96 Instrument (Roche) using the MagNA Pure 96 DNA and Viral nucleic acid (NA) Small Volume Kit (Roche) and eluted in a volume of 50 µl while using three Bioplastics processing cartridges during the first run, three Helvoet processing cartridges during the second run, and three Roche processing cartridges during de third run.
Each clinical sample and the surrounding negative samples were tested using qPCR. For all samples and adjacent samples, the target-specific-qPCR (Table 1) was used to test for contamination of known positive samples into adjacent-negative samples during the extraction process. The Cp-value of this qPCR was used to check accuracy of the cartridges compared to the Roche-cartridges. The Internal control (Target/EAV multiplex-qPCR) was used to test inhibition during extraction and amplification and to test accuracy.
For SARS-CoV-2 samples the E-gene/EAV multiplex PCR was used. E-gene primers and probe were as described by Corman et al (1). EAV primers and probe were as described by Scheltinga et al (2). Reaction conditions are described in Tables 2 and 3. EAV is used as standard internal control for the qRT-PCR to control for inhibition.
For the fecal samples the Gas1- or Gas2 (including EAV) in-house multiplex-PCR was used. Gas1 and Gas2 primers and probes are based on the primers and probes described by Svraka et al.(3)
The 10-fold dilution series of all three processing cartridges was tested in E-gene/EAV multiplex PCR, Gas1-multiplex PCR and Gas2-multiplex PCR.

***Table 2**. Reagents mixtures for SARS-CoV-2 E-gene/EAV and Gas1 and Gas2(including EAV) qRT-PCR.*

SARS-CoV-2 target qRT-PCR	µl	Gastro-target qRT-PCR	µl
4x Taqman Fast Virus MM	5	4x Taqman Fast Virus MM	5
E+EAV Mix	3	Gas1 or Gas2 Mix	3
PCR grade water	7	PCR grade water	7
Specimen nucleic acid	5	Specimen nucleic acid	5
Total volume	20	Total volume	20

***Table 3**. Amplification temperature protocol on Roche LC480 mark II thermal cycler for all q(RT)-PCR assays.*

PCR Program	Segment number	Temp Target (°C)	Hold Time (sec.)	Slope (°C/sec.)	Acquisition mode	
Reverse Transcription	1	50	900	EXTERNAL		
Denaturation/Inactivation	1	95	120	EXTERNAL		
Denaturation	1	95	60	4.4	None	LC 480
Amplification	1	95	10	4.4	None	
(cycles:50)	2	60	30	2.2	Single	
Cooling	1	40	30	4.4	None	

Results

Fit:

Details of the processing cartridges that are used for the fit test on board of the MagNA Pure96 System are shown in Table 4 for the cartridges used during three extraction-runs and in Table 5 for the cartridges used to test the fit in the drawers.

Table 4. Barcodes of processing cartridges used during extraction-run.

Barcode nr	Manufacturer	Lot nr	Positon during run
#80411190005111762	Bioplastics	1593096	left drawer
#80411190005111776	Bioplastics	1593096	middle drawer
#80411190005111763	Bioplastics	1593096	right drawer
#804010209900000328	Helvoet	production date: 29-10-2020	left drawer
#804010209900009806	Helvoet	production date: 2-11-2020	middle drawer
#804010209900000344	Helvoet	production date: 29-10-2020	right drawer
0410203000131500	Roche	20147431	left drawer
0410203000131501	Roche	20147431	middle drawer
0410203000131502	Roche	20147431	right drawer

Table 5. Barcodes of processing cartridges used during off-board leakage test and during fit-check in drawers of the MagNA Pure96 system.

Barcode nr	Manufacturer	Lot nr
#80411190005111708	Bioplastics	1593096
#80411190005111709	Bioplastics	1593096
#80411190005111710	Bioplastics	1593096
#80411190005111711	Bioplastics	1593096
#80411190005111712	Bioplastics	1593096
#80411190005111713	Bioplastics	1593096
#804010209900000329	Helvoet	production date: 29-10-2020
#804010209900000335	Helvoet	production date: 29-10-2020
#804010209900000338	Helvoet	production date: 29-10-2020
#804010209900000341	Helvoet	production date: 29-10-2020
#804010209900000343	Helvoet	production date: 29-10-2020
#804010209900009800	Helvoet	production date: 2-11-2020

All cartridges fitted well in the drawers on board of the MagNA Pure96 System. During the extraction-run the Bioplastics- and Helvoet- processing cartridges did not obstruct the needles and tips during movement.
The barcodes on the Helvoet processing cartridges were not recognized by the MagNA Pure96 System. After scanning the barcodes, a notification "barcode invalid" occurred. Therefore the barcodes were replaced by new barcodes: #80401200003012156, #80401200003012157 and #80401200003012158 were used for respectively the right, middle and left drawer. The Helvoet barcodes had the same size and position on the cartridges but had an extra digit. After changing the barcodes, the processing cartridges were run with no extra notifications.

Leakage:
The processing cartridges shown in Table 4 were checked after the extraction runs. There was no fluid visible underneath the processing cartridges. The off-board leakage-check showed no leakage after one hour of incubation. The processing cartridges used during the off-board leakage-check are shown in Table 5.

Sensitivity:
The 10-fold dilution series showed for 3 targets (NV1, NV2 and RV) the same dilutions positive comparing the three processing cartridges. The run with the Bioplastics processing cartridges missed for 2 targets (AdV and nCoV) positive results in dilution 10-4 compared to the extraction run with the Roche processing cartridges. The run with the Helvoet processing cartridges missed for 1 target (nCoV) a positive result in dilution 10-4

compared to the extraction run with the Roche processing cartridges. The non-concordant results are all for the lowest viral load samples detectable using the gold standard. Therefore, this result might be by chance due to viral load around the limit of detection of the applied PCR-tests in dilution 10-4. In Table 6 the detailed results with Cp values are shown. Across the dilutions the Cp values for the three brands of cartridges are very similar.

Table 6. *Results of the 10-fold dilution series of the combined samples shown in table 1.*

MP96-plate position	Cp NV1	Cp NV2	Cp AdV	Cp RV	Cp nCoV	measured	positive result	dilution	Manufacturer
8A	23.12	22.26	22.71	21.06	21.71	5	5	undiluted	Bioplastics
8B	27.78	26.13	26.41	25.81	26.33	5	5	10-1	
8C	32.13	28.97	29.81	30.15	30.44	5	5	10-2	
8D	36	30.59	32.8	34.86	34.7	5	5	10-3	
8E	**neg**	**neg**	**neg**	**38.59**	**neg**	5	1	10-4	
8F	neg	neg	neg	neg	neg	5	0	10-5	
8G	neg	neg	neg	neg	neg	5	0	10-6	
8H	neg	neg	neg	neg	neg	5	0	10-7	
8A	23.08	22.11	22.85	20.98	21.72	5	5	undiluted	Helvoet
8B	27.81	26.05	26.8	25.62	26.15	5	5	10-1	
8C	32.33	28.94	30.4	30.05	30.64	5	5	10-2	
8D	34.92	30.49	33.45	33.92	33.69	5	5	10-3	
8E	**neg**	**neg**	**37.78**	**37.62**	**neg**	5	2	10-4	
8F	neg	neg	neg	neg	neg	5	0	10-5	
8G	neg	neg	neg	neg	neg	5	0	10-6	
8H	neg	neg	neg	neg	neg	5	0	10-7	
8A	24.05	23.04	25.44	21.89	22.49	5	5	undiluted	Roche
8B	27.84	26.13	27.99	25.72	26.05	5	5	10-1	
8C	32.54	29	31.23	30.44	30.37	5	5	10-2	
8D	35.64	30.75	34.97	33.94	34.21	5	5	10-3	
8E	**neg**	**neg**	**37.85**	**37.65**	**36.57**	5	3	10-4	
8F	neg	neg	neg	neg	neg	5	0	10-5	
8G	neg	neg	neg	neg	neg	5	0	10-6	
8H	neg	neg	neg	neg	neg	5	0	10-7	

Contamination:

After the extraction-runs there was no fluid visible on top of the cartridges and on the deck of the MagNA Pure96 system, and no visible fluid on the drop catcher. After running the qPCRs none of the adjacent negative control samples (Figure 1) showed a positive result for the respective pathogen specific targets (Table 7). Cp values for the clinical samples between the three cartridge brands were very similar (Table 7; see also Accuracy).

Table 7. *Cp-values of the tested clinical samples, the position of the tested surrounding negative control samples and the number of positive negative control samples tested in the respective qPCRs.*

		Bioplastics	Helvoet	Roche		
Sample Name	MP96-plate position	Cp target	Cp target	Cp target	Viral target	Cartridge position of surrounding negative control samples - number of positive negative control samples
1	2B	23.58	23.82	23.27	Norovirus type 1	1A, 1B, 1C, 2A, 2C, 3A, 3B, 3C - 0
2	5G	21.6	21.15	21.3	Norovirus type 1	4F, 4G, 4H, 5F, 5H, 6F, 6G, 6H - 0
3	2E	19.5	19.49	19.63	Norovirus type 2	1D, 1E, 1F, 2D, 2F, 3D, 3E, 3F - 0
4	4E	19.84	19.74	19.68	Adenovirus	3D, 3E, 3F, 4D, 4F, 5D, 5E, 5F - 0
5	6B	18.17	18.47	18.31	Rotavirus	5A, 5B, 5C, 5A, 6C, 7A, 7B, 7C - 0
6	3G	18.73	19.24	19.25	SARS-CoV-2 virus	2F, 2G, 2H, 3F, 3H, 4F, 4G, 4H - 0
7	4B	18.83	19.47	19.5	SARS-CoV-2 virus	3A, 3B, 3C, 4A, 4C, 5A, 5B, 5C - 0
8	6E	20.83	20.65	20.27	SARS-CoV-2 virus	5D, 5E, 5F, 6D, 6F, 7D, 7E, 7F - 0

Inhibition:

159 samples were tested in the EAV-internal control PCR. The median of the EAV Cp-values from the Bioplastics-run was 26,52, from the Helvoet run was 26,49 and from the Roche-run was 26,63. The difference between both the Bioplastic-run and the Helvoet-run compared to the Roche-run were below 1 Cp.
From 157 out of 159 tested samples the EAV Cp-values were not above 1 Cp from the median Cp value. Only 2 out of 159 samples had a Cp-value higher than 1 Cp above the median. These two samples with Cp values 1.11 and 1.29 above median respectively are shown in table 8. Visual confirmation of the curves using the LightCycler-software showed that there was no clear inhibition visible.

Table 8. *Cp-values of the internal control (EAV) of the inhibited samples*

		Bioplastics	Helvoet	Roche	
Sample name	MP96-plate position	Cp EAV	Cp EAV	Cp EAV	PCR
NC 2F	2F	26.48	**27.65**	26.30	E-gene/EAV PCR
NC 7E	7E	27.12	26.65	**27.83**	E-gene/EAV PCR

In bold the outlier Cp values.

The average of 159 EAV-Cp-values of every extraction-run is shown in Table 9. The Cp-value difference between the run with the Bioplastics-processing cartridges and the Roche processing cartridges is 0.10. The Cp-value difference between the run with the Helvoet-processing cartridges and the Roche processing cartridges is 0.14. The t-test shows that the difference of Cp-values due to the use of different processing cartridges is significant, but the difference itself is small and the Cp values for Bioplastics and Helvoet are lower compared to those of Roche(Figure 2). Although the difference in Cp values is significant compared to the Cp values of the Roche-run, a lower Cp value means that there is no sign of inhibition.

Table 9. *Average Cp-values of the tested samples, t-test result and the average of the difference in Cp-value compared to the median.*

	Bioplastics	Helvoet	Roche
Internal control: EAV	Cp	Cp	Cp
Average Cp-value	26.53	26.49	26.63
Ttest: compaired with Roche	0.024	0.007	Gold standard
Average Cp-value difference	-0.01	-0.05	0.09

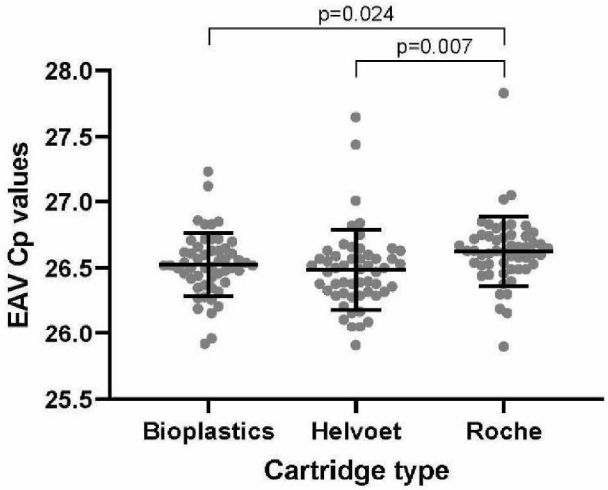

Figure 2. *EAV qRT-PCR results to determine if there are inhibiting factors present in both the Bioplastics- cartridges and the Helvoet-cartridges compared with the Roche cartridges. Shown in the figure are average (horizontal line), Standard Deviation (whiskers) and t-test results (horizontal brackets) from the comparison between the cartridge-types and the gold standard Roche.*

Accuracy

The Cp-values measured in the 10-fold dilution series (table 6) and the Cp-values of all undiluted samples (table 7) of the Bioplastics-cartridges and Helvoet-cartridges are compared with the gold standard Roche-cartridges. Differences in Cp-values (ΔCp) compared with the Roche-cartridges of all samples are less then 1 Cp except for the adenovirus 10-3 dilution (Table 10). Near the limit of detection, the precision of the measured Cp-values decreases. This taken into account, the difference in Cp-value of the adenovirus 10-3 dilution can be explained and therefore ignored.

Table 10. *Measured differences between target Cp-values of the dilution series of both the Bioplastics-run and the Helvoet-run compared to the Roche-run.*

MP96-plate position	ΔCp NV1	ΔCp NV2	ΔCp AdV	ΔCp RV	ΔCp nCoV	dilution	Manufacturer
8A	-0.93	-0.78	-0.98	-0.83	-0.78	undiluted	Bioplastics
8B	-0.06	0	-0.25	0.09	0.28	10-1	
8C	-0.41	-0.03	-0.78	-0.29	0.07	10-2	
8D	0.36	-0.16	**-1.67**	0.92	0.49	10-3	
8E				0.94		10-4	
8F						10-5	
8G						10-6	
8H						10-7	
8A	-0.97	-0.93	-0.24	-0.91	-0.77	undiluted	Helvoet
8B	-0.03	-0.08	-0.36	-0.1	0.1	10-1	
8C	-0.21	-0.06	-0.73	-0.39	0.27	10-2	
8D	-0.72	-0.26	**-1.86**	-0.02	-0.52	10-3	
8E			-0.1	-0.03		10-4	
8F						10-5	
8G						10-6	
8H						10-7	

As shown in figure 3 the differences of target Cp-values of all individual clinical samples (taken from table 7) are very small comparing both the Bioplastics-run and the Helvoet-run to the Roche-run. For all pathogens at different viral loads the difference between both Bioplastics- and Helvoet-cartridges compared to Roche-cartridges are small and therefor the results are accurate.

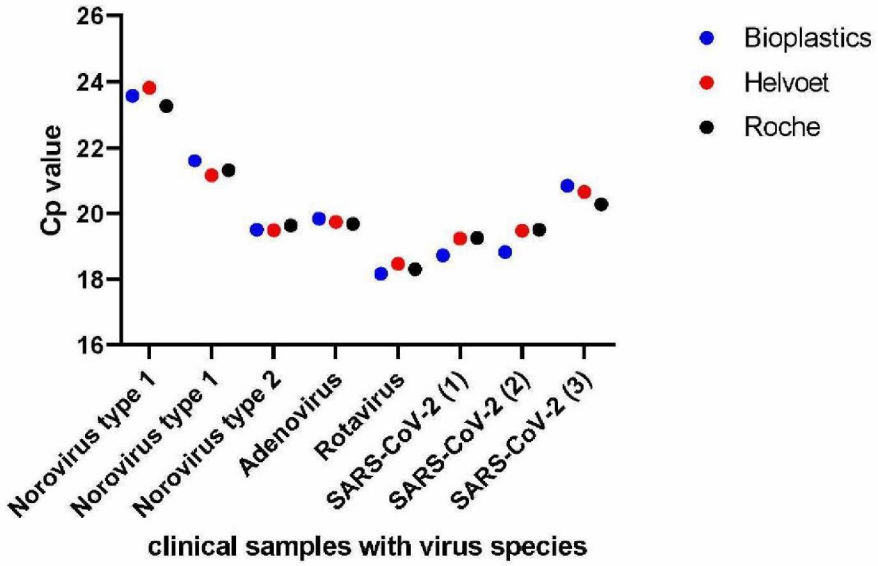

Figure 3. *Target-specific qRT-PCR results of the clinical samples to compare both the Bioplastics- cartridges and the Helvoet-cartridges with the Roche cartridges. For the different pathogens at different viral loads the Cp-values of all targets are close to the Cp-values of the Roche-run.*

Conclusion

In this evaluation both Bioplastics-cartridges and Helvoet-cartridges perform as good as the Roche-processing cartridges. All tested parameters (fit, leakage, contamination, sensitivity, inhibition and accuracy) meet the preset criteria. Therefore, the processing cartridges produced by Bioplastics and Helvoet are well suited to be used for TNA-extraction with the Roche MagNA Pure 96 system.

References

1. 5.1.2e 5.1.2e, 5.1.2e 5.1.2e 5.1.2e 5.1.2e, 5.1.2e Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3):2000045.
2. 5.1.2e 5.1.2e Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. Journal of Clinical Virology 33 (2005), 2004 Aug; 17: 306-311.
3. 5.1.2e, 5.1.2e, 5.1.2e, 5.1.2e, 5.1.2e and 5.1.2e 5.1.2e. J. Clin. Microbiol. 2009, 47(6):1674. DOI: 10.1128/JCM.00307-09