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Short report on the comparison of two new extraction kits for the isolation of viral RNA from swab samples

The aim of the experiment was to determine whether the performance of the two extraction kits PurePrep Pathogens from Molg3n / Magtivio and VIRSeek RNA Extractor from Eurofins Technologies is as good as that of the kit from Machery Nagel which currently used in routine analysis.

1. Material and methods:

1.1. Extraction kits:

1.1.1. PurePrep Pathogens from Molg3n / Magtivio

1.1.2. VIRSeek RNA Extractor from Eurofins Technologies

1.1.3. NucleoMag VET from Machery Nagel (reference)

1.2 RT qPCR Kit:

RIDAGENESARS-CoV-2 RUO (used in routine testing)

1.3. Instruments:

1.3.1. Kingfisher Flex for RNA extraction

1.3.2. Roche Lightcycler 480 II for performing RT qPCR

2. Experimental approach

To evaluate the extraction efficiency of the PurePrep Pathogens from Molgen / Magtivio and VIRSeek RNA Extractor from Eurofins Technologies Kit the PCR Positive Control of the RIDAGENESARS-CoV-2 RUO PCR Kit was used. The positive control is RNA that corresponds to a section of coronavirus RNA. It is also used in routine analysis as an extraction control. A previously unopened aliquot of this control was used.

In a 96 plate 8 positive controls (5000 RNA copies as in routine analysis) and 8 negative controls were arranged in a checkerboard pattern and extracted.

From each extract the RT qPCR was prepared with the RIDAGENESARS-CoV-2 RUO Kit in duplicates on a 96 well PCR plate. In parallel, the kit control was carried along as a PCR positive control (2500 copies as in routine analysis) and a PCR negative control was included.

The RT qPCR was performed on the Roche Lightcycler 480 II under the run designation "KF-Test-GeneScan_KF-Test-Molg3n_20200409_MKA_Casafiore" with the temperature profile protocol specified by the kit manufacturer.

The evaluation was performed with Roche Lightcycler Software 1.5.0 using the analysis module "Abs Quant/Fit Points for All Samples" (this is also used in routine analysis).

The reference values for the NucleoMag VET Kit are taken from the Roche Lightcycler 480 II run "Ireland-Test_RT_MKA_20200401_Prof".

3. Results:

3.1. Internal control (IPC):

The RIDAGENESARS-CoV-2 RUO kit contains an internal control (sequence and primers and probes used unknown). This control should show the same Cp value for all extracts and controls. Then it can be assumed that the efficiency of the PCR was equally good for all samples and controls examined in parallel. If samples show a higher Cp value, inhibition of the PCR may also be present during amplification of the viral RNA.

The following table shows the mean values of 16 measurements (Molg3n and VIRSeek) of extraction negative controls (NTC) and positive controls (RNA) as well as the PCR reaction without addition of any extract.

Furthermore, single values of the PCR positive control and the reference kit are listed.

It is shown that the IPC values for the PCR with the extracts from Molg3n Kit, Machery Nagel Kit and the PCR positive control are practically identical, in the PCR without addition of extract approx. 1.4 Cp values lower and in the PCR with the extracts from the VIRSeek approx. 1.2 Cp values higher.

This means that both the extracts and the PCR Positive Control (included in the PCR Kit) slightly inhibit the PCR, but the extracts from the VIRSeek Kit inhibit the PCR more than the extracts from Molg3n Kit and Machery Nagel Kit.

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Table1: Cp values for IPC from all kits

IPC	Meanvalue (Mv) Cp	Difference Mv (DMv) Cp testet kit to Mv Cp Machery
Cp Molg3n NTC	25,59	0,152
Cp Molg3n RNA	25,45	0,005
Cp VIRSeek NTC	26,69	1,244
Cp VIRSeek RNA	26,64	1,197
CP w/o Extract	24,01	-1,430
PCR Positive	25,32	-0,121
Cp Machery RNA	25,44	0,000

3.2. Corona RNA specific assay:

The RIDAGENESARS-CoV-2 RUO kit amplifies part of Corona E gene.

The following table shows the mean values of 16 measurements (Molg3n and VIRSeek) of extraction positive controls (RNA) as well as a single datapoint of the PCR positive control and the difference between the Cp values of the extracts. In this experiments in case of a 100 % RNA recovery by extraction the difference should be 3,33 Cp.

Table2: Cp values for RNA specific amplification from Molg3n and VIRSeek kits

RNA	Meanvalue Cp	DMv Cp to Cp PCR positive control	Expected DMv 100 % Recovery rate
Cp Molgen RNA	28,27	3,49	3,33
Cp GeneScan RNA	29,41	4,63	3,33
PCR Positive	24,78 (single data point)		

The following table shows the extraction positive controls (RNA) from Machery Nagel Kit (one data point) as well as a single datapoint of the PCR positive control and the difference between the Cp values of the extracts. Also in this experiment in case of a 100 % RNA recovery by extraction the difference should be 3,33 Cp.

Table3: Cp values for RNA specific amplification from Machery Nagel kit

RNA	Value Cp (single data points)	DMv Cp to Cp PCR positive control	Expected DMv 100 % Recovery rate
Cp Machery RNA	27,73	3,35	3,33
PCR Positive	24,38		

It is shown that the difference between the Cp values for the RNA specific PCR with the extracts from Molg3n Kit and Machery Kit and the PCR positive control is very close to the expected value for 100 % recovery.

For the VIRSeek Kit, the difference is about 1.3 Cp higher as it should be at 100 % recovery.

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4. Discussion

The data from Molg3n Kit and the Machery Nagel Kit showed that spiked RNA fragments (control RNA from RIDAGENESARS-CoV-2 RUO kit) could be recovered to almost 100 %.

As the values for IPC with extracts from VIRSeek are approx. 1.2 Cp higher as those from Molg3n Kit and the Machery Nagel Kit, and the difference between the expected Cp at 100 % recovery is about 1.3 Cp higher as it should be, it can not distinguished whether the VIRSeek kit has a lower recovery rate or inhibits the PCR stronger than Molg3n Kit and the Machery Kit or it shows a combination of both effects.

Therefore the use of the VIRSeek RNA Extractor kit for the test extraction of patient samples is not approved. Further tests must be done to bring extraction efficiency on the same level as the other two kits.

For final release of the Molg3n kit two batches of 91 samples each has be extracted successfully.

The use of the Molg3n kit for the test extraction of patient samples is therefore finally approved.

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