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Viral RNA Extraction Performance using RNAdvance Viral Supplemental Protocol

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Purpose

Viral nucleic acid extraction is the first step for downstream genomic analysis by RT-PCR. Here we present a research study at Miami Cancer Institute in collaboration with Beckman Coulter Life Sciences using the RNAdvance Viral research reagents described in the Viral Nucleic Acid extraction from swabs using RNAdvance Viral supplemental protocol. We demonstrate use of the viral RNA extracted with the RNAdvance Viral reagents (Beckman Coulter Life Sciences, RUO p/n C57956) within our RT-PCR workflow. The data presented includes the reagent's analytical performance and concordance data against a competitor's extraction kit by comparing qRT-PCR Ct values from universal transport media input material.

Workflow overview

RNAdvance Viral reagents is an RNA isolation chemistry that uses Solid Phase Reversible Immobilization (SPRI) technology. It enables the generation of highly purified RNA with demonstrated compatibility with up to 200 µL of sample input. The extraction for 24 samples processed manually takes about 1 hour. For high throughput processing, two plates (192 samples) can be run in 1.75 hours using a Biomek i5 Extraction Solution with minimal human interactions. The RNAdvance Viral workflow (Figure 1) consists of sample lysis and bind-wash-elute steps.

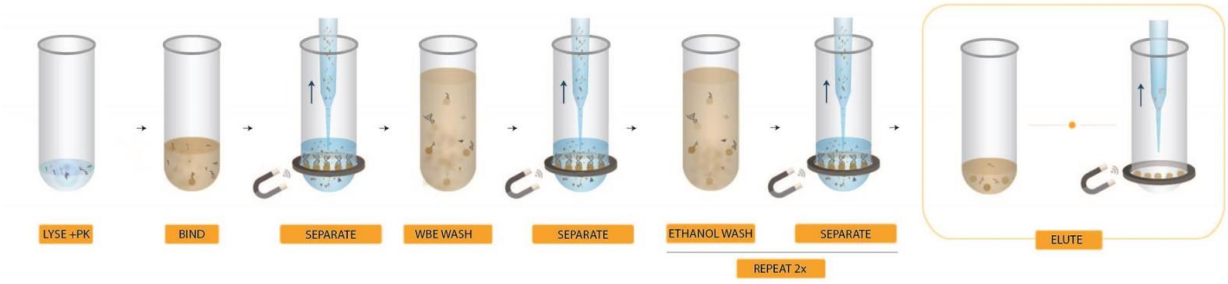

Figure 1. RNAdvance Viral Workflow Steps

Data presented was produced independently by the Miami Cancer Institute.

1. Estimation of Analytical Performance - Sensitivity

Exact Diagnostics SARS-CoV-2 Standard positive (RUO, EDX, cat# COV019) and negative (EDX, cat# COV000) controls were spiked into the pooled negative universal transport media (HEALTHLINK, cat# 330C.DHI) at 200, 20, 2, and 0.2 copies/µL. RNA was extracted manually using either Beckman Coulter RNAdvance Viral reagents or another bead-based extraction kit (Competitor A). qRT-PCR assays were carried out using Integrated DNA Technologies (IDT) 2019-nCoV RUO kit.

Ct values were assessed via qRT-PCR using research primer sets targeting the SARS-CoV-2 nucleocapsid N1, N2, and N3 fragments (N1, N2, and N3) for the detection of viral RNA and the RNase P (RP) primer set for the detection of human RNase P RNA. Positive controls 2019-nCoV_N_Positive Control (IDT: 10006625) and qPCR negative controls (NTC Ambion cat# AM4932) were included to ensure proper testing control. Exact Diagnostics RNA Standard (SARS-CoV-2 Standard) with no extraction was used for recovery efficiency control.

Data showed in the Ct value is comparable between the RNAdvance Viral reagents and the Competitor A extraction kit (Table 1). RNA that extracted using RNAdvance Viral reagents also showed consistent Ct values comparison to RNA Standard (SARS-CoV-2 Standard without extraction). RNAdvance Viral reagents demonstrate a greater than 95% recovery efficiency with no PCR inhibition. The recovery efficiency was calculated by Ct of RNAdvance Viral/Ct of RNA Standard for Primer SARS-CoV-2 N1, SARS-CoV-2 N2, and SARS-CoV-2 N3 at all viral titers.

The analytical performance is determined as the lowest viral copy concentration that shows PCR amplification. For both extractions, 0.2 copies/µL of Exact Diagnostics RNA Standard showed undetermined Ct, indicating analytical performance is between 0.2-2 copies/µL.

Sample Name	SARS-CoV-2 N1	SARS-CoV-2 N2	SARS-CoV-2 N3	RP
Competitor A_200	28.550	28.572	27.923	26.353
Competitor A_20	32.407	32.392	31.294	26.473
Competitor A_2	35.851	35.978	35.713	26.615
Competitor A_0.2	Undetermined	Undetermined	Undetermined	26.646
RNAdvance Viral_200	28.775	28.633	27.891	26.346
RNAdvance Viral_20	31.971	32.082	31.252	26.382
RNAdvance Viral_2	35.126	35.680	35.979	26.409
RNAdvance Viral_0.2	40.3239	Undetermined	38.065	26.364
RNA Standard_200	27.676	28.147	27.201	27.976
RNA Standard_20	31.178	31.530	30.527	31.472
RNA Standard_2	35.491	34.925	35.033	35.102
RNA Standard_0.2	Undetermined	37.801	36.253	37.458

Table 1. Extraction proficiency evaluation

2. Confirmation of Analytical Performance

To confirm the analytical performance (1 copy/µL) obtained from the estimation step for RNAdvance Viral reagents, RNA was extracted from Exact Diagnostics RNA Standard and spiked into the pooled negative universal transport media. The input concentration of RNA Standard is 1 and 2 copies/µL. Quadruplicate samples were run in each input concentration.

Ct value was assessed via qRT-PCR using the protocol in the Assay Set Up section described in the CDC 2019 Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel instructions (CDC-006-00019). For each sample, N1, N2, N3, and RP genes were tested. Additionally, RUO controls, 2019-nCoV_N_Positive Control (IDT: 10006625) and qPCR negative controls (NTC Ambion, cat# AM4932), were included to ensure proper testing process.

Both 1 and 2 copy/µL of RNA Standard show comparable Ct value (Table 2) and confirmed that 1 copy/µL is the analytical performance for RNAdvance Viral reagents.

Sample Name	SARS-CoV-2 N1	SARS-CoV-2 N2	SARS-CoV-2 N3	RP
RNAdvance Viral_2-1	34.443	34.892	33.656	26.408
RNAdvance Viral_2-2	34.933	35.403	34.420	26.579
RNAdvance Viral_2-3	34.627	35.489	34.272	26.405
RNAdvance Viral_2-4	35.304	34.912	34.493	26.697
RNAdvance Viral_1-1	34.314	35.901	34.785	27.612
RNAdvance Viral_1-2	35.781	36.520	36.592	27.659
RNAdvance Viral_1-3	34.966	36.637	34.827	27.611
RNAdvance Viral_1-4	35.550	34.625	35.730	27.757

Table 2. RNAdvance Viral reagents Analytical Performance confirmation

3. Sample Concordance

Sample concordance was demonstrated comparing Ct values on previously tested RNA samples extracted using Competitor A. There were 24 known positive with Ct values <40, and 23 known negative (Table 3) SARS-CoV-2. All samples were collected with a COPAN swab and stored in the HEALTHLINK universal transport media before extraction. RNA from all of the samples was extracted using RNAdvance Viral reagents manually. RT-PCR assay was performed with N2 and RP primer/probe sets. 2019-nCoV_N_Positive Control (IDT: 10006625) and qPCR negative control (NTC Ambion, cat# AM4932) were included to ensure a proper testing procedure.

The data indicates 100% concordance for all positive samples. However, 1 of 23 previously negative samples was positive, indicating 96% concordance. The operator noted it as a sample handling error.

Sample Type	Sample Number	Ct <40 No. / Total No.	Concordance No. / Total No.
Known Positive	24	24 / 24	24 / 24
Known Negative	23	1 / 23	22 / 23

Table 3. Ct Values and comparative performance of known positive and negative SARS-CoV-2 samples

Conclusion

This evaluation demonstrates a research use extraction solution for reliable isolation of viral RNA from swab samples collected in universal transport media for research. The data presented shows RNAdvance Viral extraction efficiency is greater than 95% and does not inhibit downstream qRT-PCR analysis. The analytical performance is as low as 1 copy/µL viral RNA. The detection concordance demonstrated with the known positive and negative samples is 100% and 96%, respectively, and an accuracy of about 98% compared to RNA extracted with a competitor kit Ct values. The evaluation data produced has enabled the use of the RNAdvance Viral reagents for viral RNA extraction from swab sample in universal transport media.

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