STAR BEADS Viral DNA/RNA Extraction Kit

Overview

The rapid spread of the global pandemic COVID-19 caused an unprecedented health emergency with a significant impact on public and private testing laboratories.

Several thousands of people are daily screened worldwide to find out positive cases. The increase in the number of processed samples, as well as the necessity to make a rapid diagnosis, led to a unique and immediate need for reagent supplies.

As a contribution to fighting the pandemic, Cyanagen has developed a kit for the isolation of viral RNA, which is a crucial step in the workflow of COVID-19 testing.

STAR BEADS Viral DNA/RNA Extraction Kit provides a rapid and efficient purification method to isolate high-quality viral RNA from cell-free biological fluids such serum, plasma, urine, cell-free body fluids, cell culture supernatants and rinse liquid from swabs samples.

The STAR BEADS magnetic beads technology enables the isolation of high-quality nucleic acids, ensuring the removal of potential inhibitors from sample matrices.

The purified nucleic acids are ready for direct use in downstream applications such as RT/qPCR detection as well as Next-Gen sequencing and hybridization-based.
STAR BEADS Viral DNA/RNA Extraction Kit is optimal for both standard liquid handling instruments and automated magnetic separators.
Test performed at Cyanagen demonstrated the proof of concept for using STAR BEADS Viral DNA/RNA Extraction Kit for extraction of COVID-19 RNA. Synthetic Sars-CoV-2 RNA spiked into a viral transport medium was used to mimic a clinical sample swab. The detection of viral RNA in the eluant was carried out using Real-time RT-PCR assay following Centres for Disease Control and Prevention (CDC) guidelines.

Afterwards, STAR BEADS Viral DNA/RNA Extraction Kit was validated for COVID-19 diagnostic workflow at the laboratory of U.O. Microbiologia, Laboratorio Unico del Centro Servizi, AUSL della Romagna, Pievesistina, Italy.

Description of technology

The procedure, illustrated in Figure 1, is based on the reversible adsorption of nucleic acids to the STAR BEADS magnetic beads under appropriate buffer conditions. Impurities are efficiently removed during the wash steps. First, the lysis of the sample is obtained by incubation of the sample with a lysis reagent (STAR BEADS Viral Lysis Buffer). A suspension of magnetic beads (STAR BEADS Magnetic Beads) is added to the lysate in a solution that facilitates the binding of nucleic acids to the beads. After magnetic separation, the magnetic beads are washed with two special washing reagents (STAR BEADS Washing Buffer 1 and STAR BEADS Washing Buffer 2) and finally with absolute ethanol to remove contaminants and salts. The viral RNA is then eluted with DNase/RNase free water that induces the nucleic acid to detach from the magnetic beads. The resulting high quality total nucleic acid is then ready for use in downstream applications such as RT-PCR, PCR or any enzymatic reactions or can be frozen.

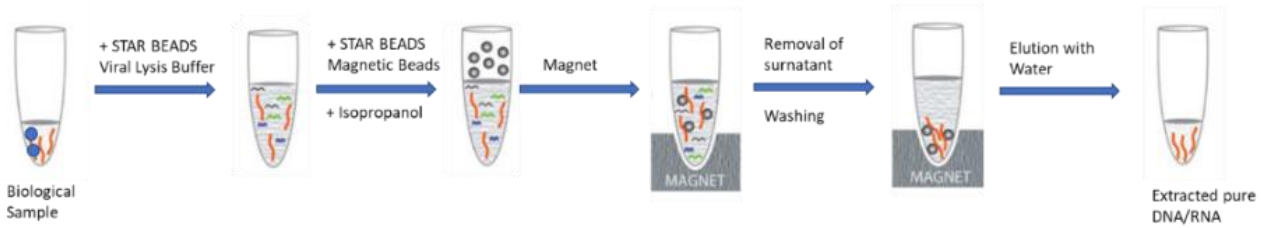

Figure 1. Nucleic acid extraction workflow with STAR BEADS Viral DNA/RNA Extraction Kit

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Limit of Detection (LoD)

A Synthetic viral RNA (2019-nCoV Positive Control, Norgen) containing two nCoV nucleocapsid target gene RNA (N1 and N2) and RNase P (internal control), was used both to assess the performance and define the approximate limit of detection of STAR BEADS Viral DNA/RNA Extraction Kit. The experimental setting mimics a clinical sample.

Viral RNA was spiked from 200000 to 0,2 copies/µL in 150 µL of Transport Medium (Vircell). RNA was manually extracted using STAR BEADS Viral DNA/RNA Extraction Kit. Elution of RNA was performed in 100 µL of nuclease-free water.

Eluted samples were assayed using Real-time RT-PCR, following the CDC protocol. Briefly, 5 µL of extracted samples per well were run on StepOnePlus™ Real-Time PCR System, Applied Biosystem in technical duplicates using TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher Scientific) and CDC 2019 Novel Coronavirus (2019-nCoV) Diagnostic Panel primers (N1, N2, and RNase P primers) in 20 µL total reaction volumes.

The analytical performance is determined as the lowest viral copy concentration that shows PCR amplification (Ct<40). The results, presented in Figure 2, demonstrate that the STAR BEADS Viral DNA/RNA Extraction Kit is robust and sensitive enough for reproducible detection of low viral titer down to 1 copy in the input sample.

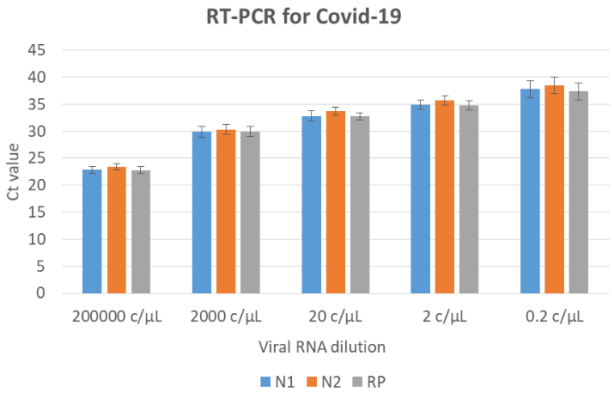

Figure 2. Limit of detection: Real-time RT-PCR detection of SARS-CoV-2 RNA down to 0,2 copies/µL in manual procedure. Ct values obtained for each of three SARS-CoV-2 specific primer sets with varying amounts of the viral synthetic RNA in the input sample as described on the graph. Values averaged from three independent experiments. Error bars represent the standard deviation.

Benchmarking

Performance of STAR BEADS Viral DNA/RNA Extraction Kit was compared with two of the most used viral RNA extraction Kits, NucleoMag Pathogen Kit (Macherey Nagel) and Quick-DNA/RNA Viral MagBead (Zymo Research).

Synthetic Sars-CoV-2 RNA was spiked at 20, 20000, and 200000 copies/µL into transport medium (Vircell) used as a diluent to mimic a clinical sample.

The viral RNA was extracted in manual procedure using STAR BEADS Viral DNA/RNA Extraction Kit and the competitors, following the manufacturer's protocol, and finally eluted in 100 µL of nuclease-free water.

Real-time RT-PCR assays were carried out following CDC protocol using TaqPath 1-Step RT-qPCR Master Mix, CG (ThermoFisher Scientific) and CDC 2019 Novel Coronavirus (2019-nCoV) Diagnostic Panel primers (N1, N2 targeting two regions of SARS-CoV-2 nucleocapsid gene and RNase P primers targeting human RNase P gene).

Data showed that the Ct values were similar in amplification of RNA extracted with the STAR BEADS Viral DNA/RNA Extraction Kit or the competitors (Figure 3).

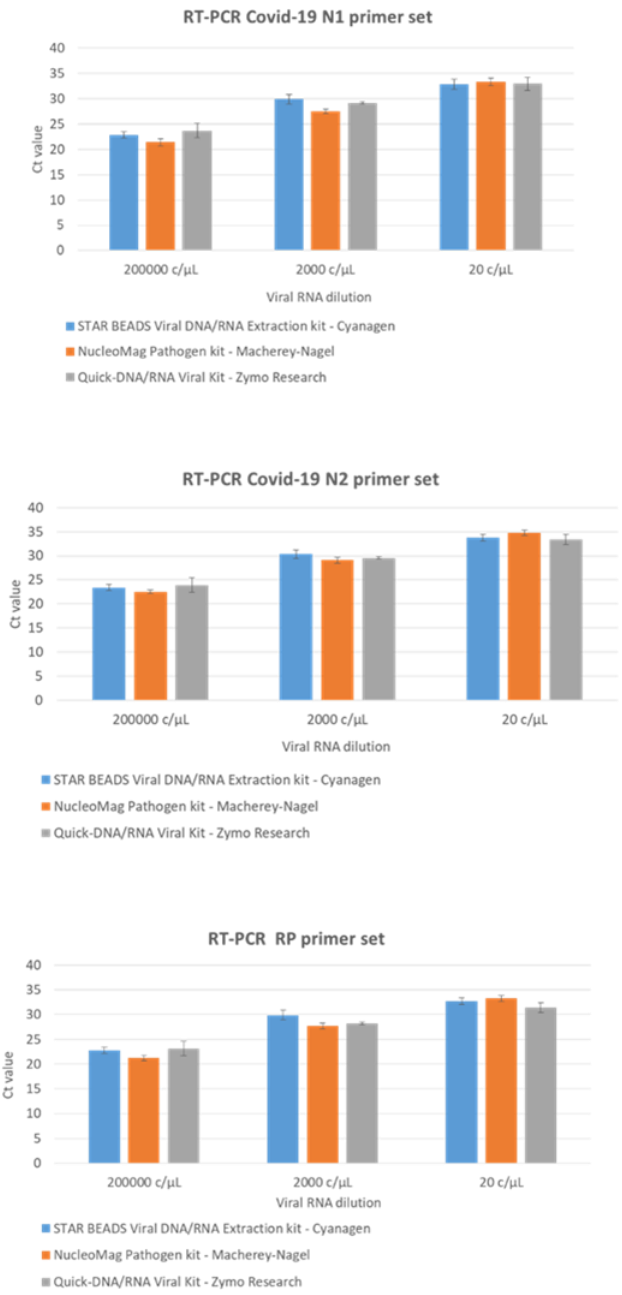

Figure 3. Real-time RT-PCR detection of SARS-CoV-2 N1, N2 and human RNase P gene using STAR BEADS Viral DNA/RNA Extraction Kit and its competitors (manual procedure).

Average Ct values obtained for STAR BEADS Viral DNA/RNA Extraction Kit, NucleoMag Pathogen Kit (Macherey-Nagel) and Quick-DNA/RNA Viral Kit (Zymo Research) on varying amounts of the viral synthetic RNA in the input sample as described on the graph. For clarity, Ct values obtained for each of the two SARS-CoV-2 specific primer sets (N1, N2) and human-specific RNase P primer set have been presented in separate graphs. Values averaged from three independent experiments; error bars represent standard deviation.

Validation for COVID-19 diagnostic workflow

STAR BEADS Viral DNA/RNA Extraction Kit was validated for RNA isolation from SARS-CoV-2 clinical samples at the laboratory of U.O. Microbiologia, Pievesestina, Italy.

For the clinical evaluation study, 166 clinical nasopharyngeal swab specimens previously tested for COVID-19 diagnosis were used. Of these 166 clinical specimens, 45 were tested positive for SARS-CoV-2 RNA, and 121 were tested negative for SARS-CoV-2 RNA.

RNA isolation was performed in parallel using STAR BEADS Viral DNA/RNA Extraction Kit and a Reference RNA Isolation Kit, routinely used for COVID-19 diagnostics in the laboratory that performed the study.

Briefly, viral RNA from 150 µL sample was extracted using STAR BEADS Viral DNA/RNA Extraction Kit with an automated procedure on Allsheng Auto-Pure96 platform. It is to be noticed that the STAR BEADS Viral DNA/RNA Extraction Kit does not need proteinase K and reducing agents for lysing of swab specimens.

In parallel, the viral RNA extraction performed with the Reference RNA Isolation Kit was carried out from 200 ul sample with an automated procedure on Nextractor® NX-48S-Genolution platform.

Elution of RNA was performed in 100 µL or 60 µL of nuclease-free water when using STAR BEADS Viral DNA/RNA Extraction Kit or the Reference RNA Isolation Kit, respectively.

Eight µL of extracted RNA was then amplified for the detection of Sars-CoV-2, using Allplex™ 2019-nCoV Assay (Seegene) for the identification of three target genes in compliance with recommendations of both Charite Medical Center and US Centers for Disease Control and Prevention.

As shown in Table 1, differences in Ct values obtained on RNA isolated with the two extraction methods are not statistically significative.

	STAR BEADS Viral DNA/RNA			Reference RNA Isolation Kit		
	E	RdRP/S	N	E	RdRP/S	N
Avg Ct value	30.90	31.47	30.53	29.82	30.51	29.17
St. Dev.	5.71	5.84	5.64	5.71	5.69	5.50

Table 1. **Comparison of Real-time RT-PCR results for STAR BEADS Viral DNA/RNA Extraction Kit and Reference RNA Isolation Kit.** Average Ct values obtained on RNA extracted from 166 SARS-CoV-19 clinical samples from nasopharyngeal swabs, using automated platforms. RNA amplified with Allplex™ 2019-nCoV Assay (Seegene). Courtesy of U.O. Microbiologia, Pievesestina.

Importantly, data demonstrated a 100% concordance between test results on sample extracted with STAR BEADS Viral DNA/RNA Extraction Kit and Reference RNA Isolation Kit. The diagnostic sensitivity and specificity were 100%.

The Sample concordance is demonstrated comparing Ct values on viral RNA extracted with STAR BEADS Viral DNA/RNA Extraction Kit and Reference RNA Isolation Kit. The data shown in Figure 4, indicates 100% concordance for both the positive and negative samples. Therefore, the STAR BEADS Viral DNA/RNA Extraction Kit performance can be considered comparable to the extraction methods currently used in routinary testing at U.O. Microbiologia, Pievesestina.

KIT	Reference RNA Isolation Kit (U.O. Microbiologia, Pievesestina)			
STAR BEADS Viral DNA/RNA Extraction Kit		+	-	Total
	+	45	0	45
	-	0	121	121
	Total	45	121	166

Figure 4. Concordance between test results obtained with STAR BEADS Viral DNA/RNA Extraction Kit and the Reference RNA Isolation Kit for COVID-19 diagnostics. STAR BEADS Viral DNA/RNA Extraction Kit has been validated for RNA isolation from SARS-CoV-2 clinical samples on 166 samples (45 positive samples and 121 negative samples) from nasopharyngeal swabs. RNA isolation was performed in parallel using STAR BEADS Viral DNA/RNA Extraction Kit and a reference Kit at U.O. Microbiologia, Pievesestina. RNA amplified with Allplex™ 2019-nCoV Assay (Seegene). Courtesy of U.O. Microbiologia, Pievesestina.

Moreover, STAR BEADS Viral DNA/RNA Extraction Kit provides highly reproducible viral RNA isolation. Results for a viral RNA-based internal positive control show a coefficient of variation (CV%) within runs (intra-assay) and between runs (inter-assay) respectively of 3,02 and 3,44%, demonstrating the robustness of the method.

	Reproducibility		
	Mean Ct	St. Dev.	CV %
Intra-assay variability	25,30	0,76	3,02
Inter-assay variability	25,93	0,89	3,44

Figure 5. Reproducibility of test results obtained with STAR BEADS Viral DNA/RNA Extraction Kit for COVID-19 diagnostics. Mean Ct, Standard deviation and Coefficient of Variation (CV%) of test results obtained on a viral RNA-based internal positive control on RNA extracted with STAR BEADS Viral DNA/RNA Extraction Kit from nasopharyngeal swabs clinical samples. RNA amplified with Allplex™ 2019-nCoV Assay (Seegene). Courtesy of U.O. Microbiologia, Pievesestina.

Conclusion

In response to global events related to COVID-19 disease, Cyanagen has worked rapidly to develop a kit for the isolation of viral RNA to support increased SARS-CoV-2 testing throughput.

STAR BEADS Viral DNA/RNA Extraction Kit provides an efficient and reproducible method for nucleic acid extraction of viruses from cell-free biological fluids with no need of Proteinase K and reducing agent treatment, thus offering a fast and simple protocol. STAR BEADS Viral DNA/RNA Extraction Kit has been validated for Sars-CoV-2 RNA isolation from clinical respiratory samples.

The extraction methodology is easily adaptable to automated systems, such as liquid handling instruments or automated magnetic separators.

STAR BEADS Viral DNA/RNA Extraction Kit provides an excellent limit of detection results and highly reproducible viral RNA extraction.

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