APPLICATION NOTE

Automated Nucleic Acid Extraction for Pathogen Detection using ROBOSCREEN's **Rapid INSTANT Virus RNA/DNA Kit - FX** on Hamilton **STAR V**

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Introduction

Molecular testing of clinical samples for viral pathogens has already long become standard in both research and diagnostic laboratories. Every molecular biological test relies on high-quality sample material to achieve the best possible sensitivity and specificity. Therefore, the first step of sample analysis – the extraction of viral RNA and DNA – is of the utmost importance for the final result. Laboratories with high-sample numbers require automated extraction systems that enable high-throughput analysis with a minimum of manual interventions, reducing the risk of errors and ensuring high repeatability.
The diversity of clinical sample sources poses additional challenges. Additionally, flexible kits that can be applied for automated extraction from various starting material elevate the challenges for laboratory staff.

- Improve your assay sensitivity by achieving better primary extract quality
- Fast turnaround time – from 96 samples to eluates in one hour
- Save money and preserve the environment by reducing tip and plate consumption

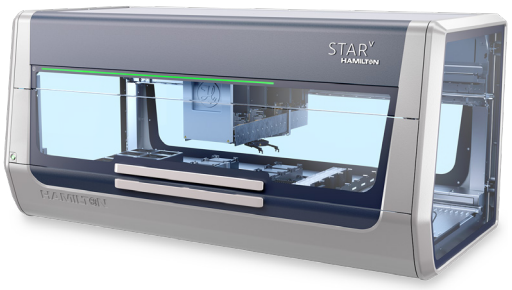


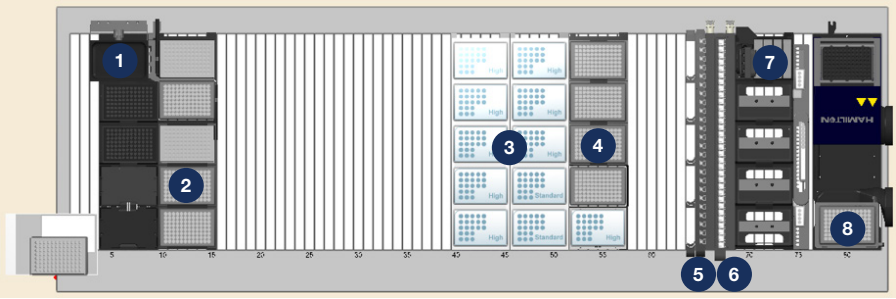
Figure 1: The Hamilton STAR V Platform.

Method Description

The CE-IvD labeled kit Rapid INSTANT Virus RNA/DNA Kit – FX (ROBOSCREEN, #847-0259200906) is intended for automated nucleic acid extraction in combination with the liquid handling platform Cybio FeliX. The kit uses magnetic particle technology for efficient extraction ensuring high yields of ultra-pure nucleic acids in a minimal processing time. The kit can be applied to diverse sample types such as blood, swabs, stool, and others. The starting sample volume is 400 µL and the elution volume can be defined in a range between 50 and 200 µL (tested with 100 µL in this case). The protocol and the components of the kit have been used to establish a method for nucleic acid extraction on the Microlab STAR V platform (Fig. 1).

This Application Note compares results obtained on Hamilton STAR V, with results generated by a routinely used automated extraction method (competitor platform). Here, the isolated nucleic acids were used for the detection of several respiratory pathogens with one and the same qPCR assay, which detects up to 22 different pathogens.

System Description



1. Gravity Waste
2. 3x Plate Stackers
3. Tip Carrier
4. Carrier for Deep Well Plates with Tip Park Position and Magnetic Stand
5. Trough Carrier
6. Cooling Carrier
7. Quad Core Gripper and Trough Position
8. Inheco Thermoshake AC with DWP Adapter

Figure 2: Deck Configuration of the STAR V System for Automating the ROBOSCREEN Rapid INSTANT Virus RNA/DNA Kit - FX.

Technology

The STAR V platform enables parallel extraction of up to 96 samples in minimal time and is individually adaptable due to its modular open design. The method uses the 96 Multi-Probe Head for simultaneous pipetting, significantly reducing processing times and column-based effects. Additionally, the method utilizes the capacitive Liquid Level Detection (cLLD) technology of Hamilton CO-RE tips.

Figure 3: A Protective Plate (Included in the ROBOSCREEN Plate set - INSTANT FX / HMT) allows tips used for buffer transfers to drip-off, which can thereafter be reused to reduce plastic waste.

Figure 4: The components of the Rapid INSTANT Virus RNA/DNA Kit - FX

Visual Workflow

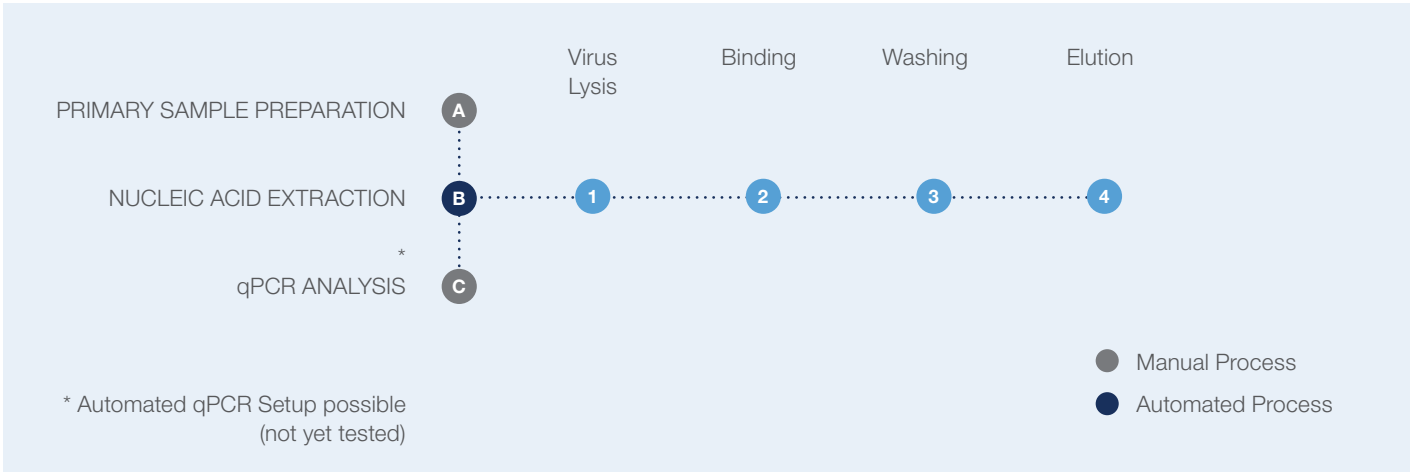


Figure 5: Visual Workflow Representation of the ROBOSCREEN Rapid INSTANT Virus RNA/DNA Kit - FX with Subsequent qPCR Analysis.

Validation

Buccal swab samples were prepared and processed on the STAR V, according to the Instructions for Use of the Rapid Instant FX protocol (Rev. 0_10 / 2022). Prefilling of lysis buffer and Proteinase K are followed by sample addition and incubation on the Thermoshake AC. During incubation of the lysates, binding buffer and magnetic beads are prefilled. Wash buffers are directly pipetted into individual sample wells from reagent troughs, which reduces the number of Deep-Well Plates, as compared to the standard protocol.
Two sets of different samples were extracted on separate days (Run1: n=26, Run2: n=21).
After Isolation, samples were processed according to Siemens' FTD Respiratory pathogens 21 and FTD SARS-CoV-2 qPCR assays. qPCR reactions were performed on a Biorad CFX Real-Time PCR Detection System. Fluorescence thresholds were set as follows; CY5: 700 RFU, HEX: 900 RFU, ROX: 1500 RFU and FAM: 1300 RFU.

Results

Compared to the results obtained with DNA/RNA extracted on the competitor's automation platform, some of the Ct values of true positive results were improved, indicating higher sensitivity. Furthermore, 11 assay targets were missed in nine samples extracted on the competitor's platform (i.e. false negative), while those targets were clearly identified in the samples extracted on the STAR V (Tables 1 and 2). Note that in clinical routine, Ct values higher than 35 are interpreted as inconclusive and could be the result of unspecific amplification. In such cases viral load must be assumed to be very low and the sample needs to be investigated by alternative approaches.

Table 1: qPCR results of Siemens' FTD Respiratory Pathogens 21 and FTD SARS-CoV-2 Assays performed on samples extracted on the Hamilton STAR V or a competitor's platform. Two separate extraction runs were analyzed. Abbreviations are explained in the legend below. Ct Values for individual pathogen targets are indicated. (A) Run 1 with a Total of 26 samples (B) Run 2 with a Total of 21 samples.

A

Run1, n=26				
	Hamilton		Competitor	
Sample	Detected pathogen(s)	Ct value(s)	Detected pathogen(s)	Ct value(s)
1	none	-	none	-
2	none	-	none	-
3	RHV	35.43	none	-
4	none	-	none	-
5	AV BocaV	27.53 38.16	AV none	27.30 -
6	Para4	29.9	Para4	30.90
7	RHV	34.52	RHV	38.80
8	none	-	none	-
9	none	-	none	-
10	EV	37.61	none	-
11	RHV AV	27.81 35.26	RHV AV	34.00 33.60
12	none	-	none	-
13	none	-	none	-
14	none	-	none	-
15	none	-	none	-
16	AV	26.48	AV	27.30
17	none	-	none	-
18	RHV	32.43	RHV	35.60
19	RHV	32.63	RHV	36.60
20	AV	26.49	AV	22.30
21	none	-	none	-
22	none	-	none	-
23	P	24.38	P	20.81
24	none	-	none	-
25	none	-	none	-
26	none	-	none	-

B

Run2, n=21				
	Hamilton		Competitor	
Sample	Detected pathogen(s)	Ct value(s)	Detected pathogen(s)	Ct value(s)
1	none	-	none	-
2	EV	29.9	EV	33.7
3	Para4	35.96	Para4	35.96
4	Cor43	36.62	none	-
5	none	-	none	-
6	RHV EV AV	37.40 37.28 33.73	RH4 none AV	39.8 - 29
7	FLUA H1N1	28.27 29.22	FLUA H1N1	28.94 26.77
8	none	-	none	-
9	none	-	none	-
10	EV RHV AV	29.11 30.95 38.75	EV none none	35.21 - -
11	EV RHV AV	37.93 36.13 25.48	none none AV	- - 25.78
12	Para4	33.16	Para4	35.82
13	none	-	none	-
14	none	-	none	-
15	EV	37.61	none	-
16	RHV	28.19	RHV	31.23
17	none	-	none	-
18	RHV	35.78	none	-
19	none	-	none	-
20	none	-	none	-
21	none	-	none	-

Legend:
RHV: Rinovirus, AV: Adenovirus, BocaV: Bocavirus, Para4: Parainfluenzavirus 4, EV: Enterovirus, Cor43: Coronavirus 43, FluA: Influenza A, P: Parechovirus, H1N1: Influenza A Subtype

Assay Result	n (Hamilton)	n (Competitor)
true positive	30	19
true negative	25	25
false negative	0	11
Total	55	55

Table 2: Summary of results, based on individual assay targets. 11 assay targets were not identified in samples extracted with a competitor's automation platform.

Throughput and Capacity

Sample Number	Duration (Hamilton)	Duration (Competitor)
48	50 min	59 min
96	1 h	1h 15 min

Summary

The most important criteria for labs are the performance quality of testing, the reporting time, and the costs. Here we have demonstrated that, using the automated protocol and the components of the ROBOSCREEN Rapid INSTANT Virus RNA/DNA Kit – FX on the Hamilton STAR V platform, delivers high-quality sample extracts delivering high sensitivity in downstream qPCR analysis. Further, the fast processing of more samples drastically improves turnaround time, which can be additionally improved by automated qPCR setup.
Last but not least, reducing the amount of consumables by the ability to reuse pipette tips has not only a positive effect on material costs, but also reduces the environmental impact by reducing plastic waste.

Requirements

Deck Hardware Component	Provider	Quantity
MPH Liquid Waste	191008 / Hamilton	1
MFX Stacker Module	188044 / Hamilton	3
Tip Carrier	182085 / Hamilton	2
MFX DWP Module	188042 / Hamilton	3
MFX DWP Module XXL	188293 / Hamilton	1
MFX Tip Park Module for reusable Tips	188402 / Hamilton	1
Ambion Magnetic Stand 96	282661 / Hamilton	1
Reagent Carrier	194057 / Hamilton	1
Cooling Carrier Module Or Sample Tube Carrier	10066532 / Hamilton Or 173410 / Hamilton	1 1
Inserts for 2mL	188102 / Hamilton	1
Quad CO-RE Gripper	96006-01 / Hamilton	1
MFX RGT QCG Module	10073128 / Hamilton	1
Inheco Thermo Shake AC	7100160 / Inheco	1
Adapter S Roboscreen	3200823 / Inheco	1

Consumables	PN / Provider	8 Samles	48 Samples	96 Samples
1000 µL CO-RE Tips	235905 / Hamilton	48	88	136
300 µL CO-RE Tips	235903 / Hamilton	32	72	120
Rapid INSTANT Virus RNA/DNA Kit - FX	847-0259200906 / ROBOSCREEN	1	1	1
Plate set - INSTANT FX / HMT	847-0259200938 / ROBOSCREEN	1	1	1
60 mL Troughs	56694-01 / Hamilton	5	5	5
20 mL Troughs	96424-02 / Hamilton	2	2	2

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