Molecular Controls for SARS-CoV-2 and Influenza A/B Testing in Microchip-based RT-PCR Test Systems

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Summary

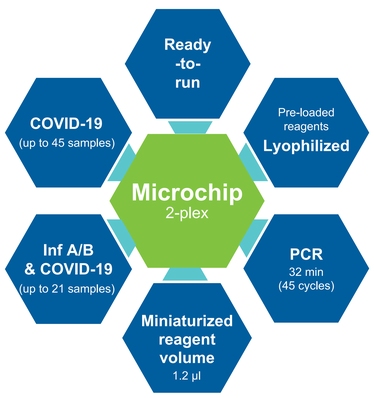
- Seasonal respiratory viral pathogens can often be challenging to distinguish from one another symptomatically. For accurate and early detection of these viruses, extremely sensitive molecular detection methods like real-time PCR demand availability and inclusion of standard RNA positive controls in the test system.
- Here we describe a robust, sensitive, accurate, and cost-effective microchip-based Influenza A, Influenza B, and SARS-CoV-2 rapid detection system that utilized Armored RNA Quant® controls (Asuragen, a Bio-Techne Brand, TX) to achieve these objectives.
- These molecular controls were also used for quality control checks of ready-to-use microchips with pre-loaded and lyophilized reagents, as positive extraction controls, and for determining technical limit of detection (LoD).

Introduction

Why are RT-PCR and RNA molecular controls required?

- Accuracy of SARS-CoV-2 testing determines
  - » If someone is infected with SARS-CoV-2
  - » The need for patient quarantine and/or treatment
- Real-time RT-PCR assays are the tool of choice
  - » Extremely sensitive
  - » Need only trace amount template RNA
- Molecular controls are required
  - » Aid in the interpretation of results
    - Identify contamination during processing
    - Inhibition of the reverse transcription
    - Inhibition of amplification reactions
  - » Confirm the success of RNA extraction

Why is microchip-based RT-PCR preferred?



What quality controls are needed?

- Negative template control (NTC)
- Positive template control (PTC)
- Negative extraction control (NEC)
- Positive extraction control (PEC)
- Internal control (IC)

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What characteristics do ideal molecular controls offer?

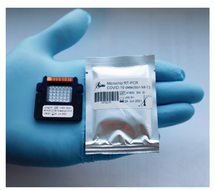
Characteristic	Objective	Armored RNA Quant®
Safe from any pathogenicity	To minimize biohazard concerns	• *In vitro* transcribed RNA • Non-infectious
Stability	• Free from degradation • Long-term stability • Long-term functionality	• Encapsulated in a protein coat as virus-like particle • Resistant to degradation
Reliable sourcing	• Product reliability • Molecular authenticity	• Standardized • Quality manufacturing • Reliable and consistent
Target gene specificity	• SARS-CoV-2: Nucleocapsid • InfA: Matrix gene • InfB: Nonstructural 2 gene	Yes
Adequate concentration	• To allow 10X dilution series • Determining linearity • Determining LoD	Offers $1x10^{11}$ or $1x10^{8}$ copies/mL
Storage	• Thermal stability • Cost effective • Cold chain-free transport	Require storage at -15 to -30°C versus -70°C
Bottleneck-free supplies	Uninterrupted supply	No supply bottleneck during pandemic encountered
Lyophilization compatibility	Compatibility for lyophilization in the microchip	Under development
Minimal upstream processing	• Ready useability in testing • Minimal handling steps	• Manufacturer: heating at 75°C x 3 min • Pre-heating not necessary*
Interference and contamination	• Assay compatibility • No detrimental impact	Yes

*Ragan *et al.* Comparison of media and standards for SARS-CoV-2 RT-qPCR without prior RNA preparation. medRxiv [Preprint]. 2020 Sep 17:2020.08.01.20166173. doi: 10.1101/2020.08.01.20166173.

Materials and Methods

Microchip prep:

- Pre-loaded and lyophilized primer and probe sets of InfA/InfB, and N1/HsRP30 targets as 2-plex
- The microwell array in the microchip (5 row x 6 column = 30 or 6 row x 8 column = 48) formats



Ready-to-run microchip

Asuragen's Armored RNA Quant (ARQ)*

- Influenza (P/N 52013)
- SARS-CoV-2 (P/N 52030)
- RNase P (P/N 52031)
- SARS-CoV-2 Panel (P/N 52036)
- Respiratory Triplex Control (P/N 52108)

Applications

- Quality control (QC) checks of the microchip production
- As positive test controls (PTC)
- Performing PCR on AriaDNA™ Analyzer
- Technical LoD
- Reproducibility studies:
  - » Microchip manufacturing site
  - » Clinical sample testing site

AriaDNA™ Analyzer

Results

For a QC check:

- The Armored RNA Quant Influenza, SARS-CoV-2, and RNase P were applied as PTC onto the entire microchip generating Ct values within acceptable ranges of Ct 29 ± 2 for InfA/InfB and Ct 28 ± 2 for N1/HsRPP30.
- The resulting intra-chip CV% of Ct values among replicates (n=15) for each target was highly reproducible with CV% of 0.4/0.4 and 0.1/0.2, respectively.
- The inter-chip variability of Ct values, and the qualitative scores reported as +ve or -ve by the analyzer indicated that the Armored RNA controls worked well to reflect the quality of the lyophilized reagents in the microchips.

Technical LoD:

- The LoD of Armored RNA Quant SARS-CoV-2, and Armored RNA Quant RNase P determined to be 1 copy/PCR for N1 [Gill *et al.* 2021]
- Supported by Razvan *et al.*, 2021 and Campos-Stairiker *et al.*, 2021 obtaining LoD of 1 and 3 copies/PCR for N1, respectively using qPCR Control RNA from Heat-Inactivated SARS-Related Coronavirus 2, Isolate USA-WA1/2020 Catalog No. NR-52347.
- Results encouraged the use of Armored RNA as a QC tool for microchips and as PTC in the clinical test.

Clinical sample testing:

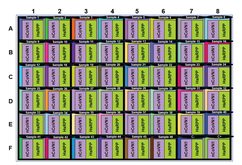
- At the clinical sample testing facility, the Armored RNA Quant Influenza and SARS-CoV-2 were used as PTC along with samples loaded onto the test microchip.
- Seventeen randomly picked RT PCR runs resulted in CV% of 5.4/6.59 and 2.34/2.63 for InfA/InfB and N1/HsRPP30 targets, respectively.
- Results indicated acceptable stability and reproducibility of the Armored RNA controls.

As a solution to supply-bottlenecks:

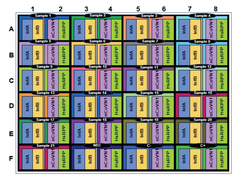
- Due to the recent pandemic situation, Armored RNA Quant Respiratory Triplex Control (P/N 52108) that included all four targets (InfA/InfB/SARS-CoV-2-N1/HsRPP30) was also studied.
- It resulted an CV% of 0.3/0.7 and 0.1/0.2 of the Ct values across 5 replicates of the respective targets.

Table 2. Clinical Sample (Microchip # 007QU75)

#	Rn file #	InfA	InfB	N1	HsRP
1	2043	28.63	27.94	26.68	25.97
2	1340	30.21	29.55	27.24	26.65
3	122-CD	30.59	29.65	26.7	26.83
4	122-AK	28.96	28.68	26.15	26.1
5	1604	29.13	28.73	26.84	26.33
6	1608	28.24	27.83	26.54	25.93
7	1032	33.28	32.83	26.83	26.64
8	1404	33.15	32.99	26.18	25.33
9	1513	33.49	33.17	26.2	26.65
10	1045	31.69	31.69	26.3	25.72
11	1437	31.94	31.81	26.45	26.54
12	1711	31.99	31.72	26.19	25.37
13	1800	30.44	34.18	24.52	27.36
14	2030	32.95	33.81	26.1	25.15
15	948	31.95	31.91	26.11	25.33
16	1230	31.67	32.04	26.37	25.86
17	1402	31.03	30.61	25.34	24.91
	Mean	31.14	31.13	26.28	26.04
	SD	1.7	2.1	0.6	0.7
	CV%	5.40	6.59	2.34	2.63



Test panel: SARS-CoV-2 N1, HsRPP30 (IC). Analyses 45 patient samples (Microchip kit # 007QO69)



Test panel: InfA, InfB, SARS-CoV-2 N1), and HsRPP30 (IC). Analyzes 21 patient samples (Microchip kit # 007QU75)

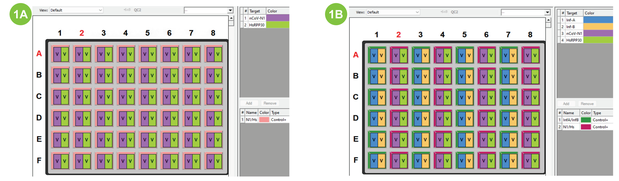


Figure 1. Qualitative Results of the Application of Armored RNA. A) Quant SARS (CoV-2) $1x10^3$ copies/PCR in QC check of COVID-19 microchips, and **B)** Quant Respiratory Triplex $1x10^3$ copies/PCR for QC check of Influenza A, B and COVID-19 (ABC) microchip.

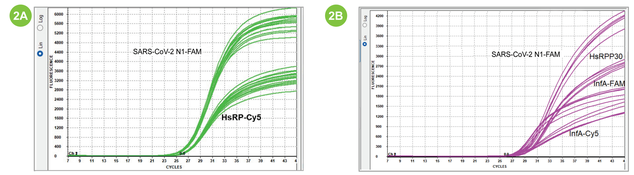


Figure 2. Semi-quantitative Results of the Application of Armored RNA. A) Quant SARS (CoV-2) $1x10^3$ copies/PCR in QC check of COVID-19 microchips, and **B)** Quant Respiratory Triplex $1x10^3$ copies/PCR for QC check of Influenza A, B and COVID-19 (ABC) microchip.

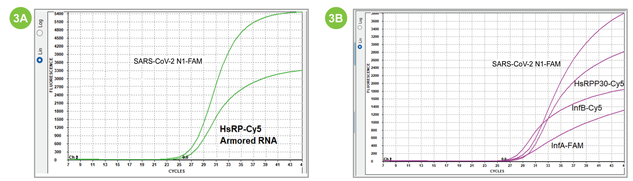


Figure 3. Application of Armored RNA as PTC. A) Quant SARS (CoV-2) $1x10^3$ copies/PCR in COVID-19 microchips, and **B)** Quant Respiratory Triplex $1x10^3$ copies/PCR in Influenza A, B and COVID-19 (ABC) microchip.

Conclusions

The characteristics of Armored RNA Quant® including resistance to nuclease degradation, safety being non-infectious in nature, and availability at high titer, contributed to the success of the launch of AriaDNA™ microchip test system.

Acknowledgments

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