Feasibility of a Single-Well HDPCR™ Flu A / Flu B / RSV / SARS-CoV-2 PCR Assay for High-Throughput Testing

Introduction

The upcoming 2020 – 2021 respiratory season will be the first in which an active pandemic agent, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), will overlap with circulation of traditional pathogens like influenza and respiratory syncytial virus (RSV) for a full season. While the initial impact of SARS-CoV-2 in the United States occurred during the tail end of the 2019 – 2020 respiratory season, first hitting the United States between December 2019 and January 2020, there was not sufficient time to fully study the co-infection rate of SARS-CoV-2 with other common respiratory pathogens. The few analyses published suggest the co-infection rates of common respiratory pathogens with SARS-CoV-2 range from 3% - 20%. [1,2] This preliminary data hints at the importance of being able to simultaneously test for the presence of SARS-CoV-2 with other common respiratory pathogens in a single, high-throughput test. This white paper discusses feasibility testing with the novel HDPCR Flu A / Flu B / RSV / SARS-CoV-2 (COVID+) assay, a single-well, high-throughput PCR test for some of the most common respiratory pathogens from upper respiratory sample types in order to better understand the epidemiology of these viruses.

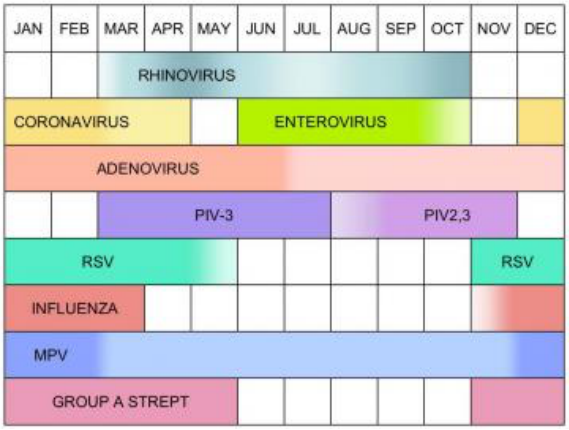

Figure 1. Seasonality of common respiratory viruses[3] (SARS-CoV-2 not included in figure)

Methods & Materials

HDPCR Overview: ChromaCode's HDPCR multiplexing technology empowers the global installed base of qPCR instruments to perform multiplex testing at a very low cost with no instrument or software modifications required. HDPCR couples traditional chemistry (TaqMan®) with proprietary data-science based algorithms to re-engineer the qPCR curve by significantly reducing unwanted variability from chemical and/or sample factors as well as thermal and optical variability from qPCR hardware. Within this controlled system, ChromaCode is able to then encode multiple analytes into a single color channel and differentiate targets by varying end-point signal intensity for a given target. All HDPCR tests have an identical workflow to traditional qPCR assays and utilize ChromaCode's HIPAA compliant ChromaCode Cloud software (**Figure 2**).

Figure 2. The COVID+ test workflow is identical to traditional qPCR workflow for frictionless integration into lab workflow

COVID+ Test Design: COVID+ is a semi-quantitative qPCR assay that detects influenza A, influenza A subtype H1, influenza A subtype H3, influenza B, RSV A, RSV B, and SARS-CoV-2 in a single reaction well from common upper respiratory sample types in universal and viral transport media (UTM/VTM). The test is intended to be compatible with common automated nucleic acid extraction platforms like the Thermo Scientific™ KingFisher™ System, BioMerieux easyMag® and Emag® systems, and Roche MagNA Pure system and most Thermo Fisher qPCR instruments. COVID+ can be run on both 96- and 384-well formats (**Table 1**).

The current test configuration utilizes four color channels and separates virus targets in a single channel using a binary multiplexing scheme. The four COVID+ test reportables are influenza A, influenza B, RSV (A&B), and SARS-CoV-2, with information on influenza A subtype and individual targets for SARS-CoV-2 available in the detail report. The influenza A, influenza A subtype H1, and influenza A subtype H3 are in the FAM channel, SARS-CoV-2 N1 gene and N2 gene targets are in the HEX channel, RSV A and RSV B are in the ROX channel, and influenza B and the internal control (RNase-P) are in the CY5 channel (**Figure 3**). SARS-CoV-2 is deemed detected if the N1 gene or N2 gene is detected.

Sample Type Compatibility	⇒	Compatible with common upper respiratory sample types
Extraction Instrument Compatibility	⇒	Compatible with most automated nucleic acid extraction platforms
qPCR Instrument Compatibility	⇒	Applied BioSystems™ 7500 Fast Applied BioSystems™ 7500 Fast Dx Applied BioSystems™ QuantStudio 12K Flex Applied Biosystems™ QuantStudio 7 Applied BioSystems™ QuantStudio 5 Applied BioSystems ViiA7
Run Time	⇒	< 2.0 hours
Throughput	⇒	96-well plate: ~90 samples per run 384-well plate: ~378 samples per run
Kit Format	⇒	96 and 384 test kit configurations Positive control Negative control

Table 1. Key specifications of the HPDCR COVID+ assay. COVID+ is currently in development and the design is subject to change.

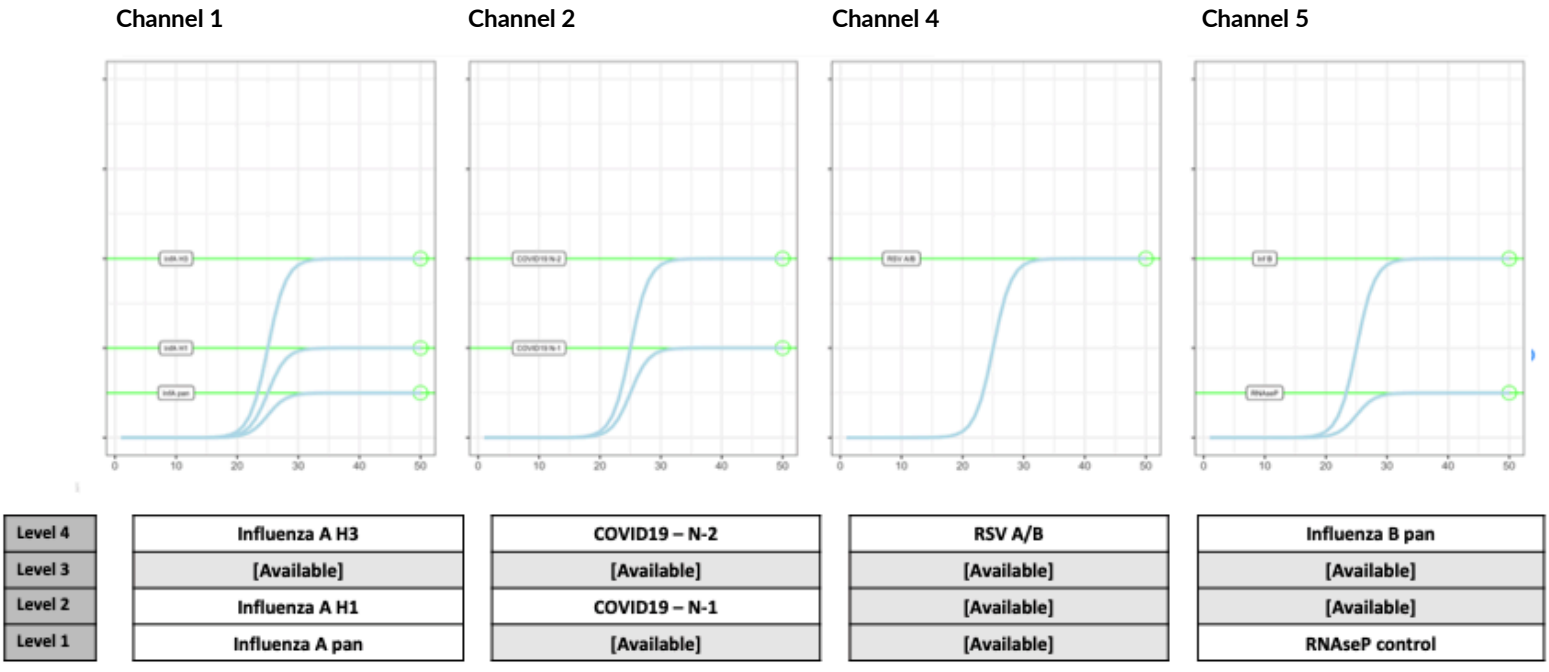

Figure 3. Current layout of the multiplex, single-well COVID+ assay

Comparison Study: A comparison study was performed using residual nasopharyngeal (NP) swabs specimens in UTM/VTM that were previously tested with the FilmArray Respiratory Pathogens 2 Test (BioMerieux, Marcy-l'Etoile, France) and/or Cepheid Xpert Flu test (Cepheid, Sunnyvale, CA) and Armored RNA SARS-CoV-2 (Asuragen, Austin, TX) spiked into negative NP matrix between 2,000 copies/mL and 30,000 copies/mL. Nucleic acid extraction was performed on the MagNA Pure 24 System (Roche, Basel, Switzerland) using the RNA Isolation Kit, with 200 µL of sample being eluted to 50 µL. Real-time PCR testing was performed on the Applied BioSystems QuantStudio 5 real-time PCR system (Thermo Fisher, Waltham, MA). Results were analyzed on ChromaCode's cloud-based software ChromaCode Cloud. Statistical analysis was completed using http://vassarstats.net/clin1.html.

Results

A total of 62 residual NP swab specimens and 10 contrived NP swab specimens were tested with the preliminary COVID+ panel, including 27 influenza A samples, 20 influenza B samples, 9 RSV samples, and 10 SARS-CoV-2 samples. COVID+ had a 100% positive percent agreement and negative percent agreement (**Table 2**). For the 27 influenza A samples tested, COVID+ correctly subtyped these strains as H1 (n = 16) or H3 (n = 11).

Target	Correlation: Positives	Correlation: Negatives	PPA (95% CI)	NPA (95% CI)
Influenza A	27/27	45/45	100% (84.5% - 100%)	100% (90.2% - 100%)
Influenza A Subtype H1	16/16	56/56	100% (75.9% - 100%)	100% (92.0% - 100%)
Influenza A Subtype H3	11/11	61/61	100% (67.9% - 100%)	100% (92.6% - 100%)
Influenza B	20/20	52/52	100% (80.0% - 100%)	100% (91.4% - 100%)
RSV (A&B)	9/9	63/63	100% (62.9% - 100%)	100% (92.8% - 100%)
SARS-CoV-2	10/10	62/62	100% (65.6% - 100%)	100% (92.7% - 100%)
Total	**93/93**	**339/339**	**100% (95.1% - 100%)**	**100% (98.6% - 100%)**

Table 2. Preliminary performance of the COVID+ assay from a small method comparison study (n = 72) performed during development

Visualization of Results in ChromaCode Cloud: ChromaCode Cloud provides an extremely user-friendly interface, allows for automated data upload from the most common qPCR instruments, streamlines data analysis and quality control for users, and provides a fully secure environment that is both HIPAA compliant and does not store protected health information (PHI). The ChromaCode Cloud software experience rivals that from the best software on the simplest sample-to-answer molecular platforms. ChromaCode Cloud also provides users with the unique ability to customize multiple testing feature to optimize testing performance to meet institution-specific requirements. **Figure 4** below highlights the simplicity in the plate summary and well summary pages in ChromaCode Cloud.

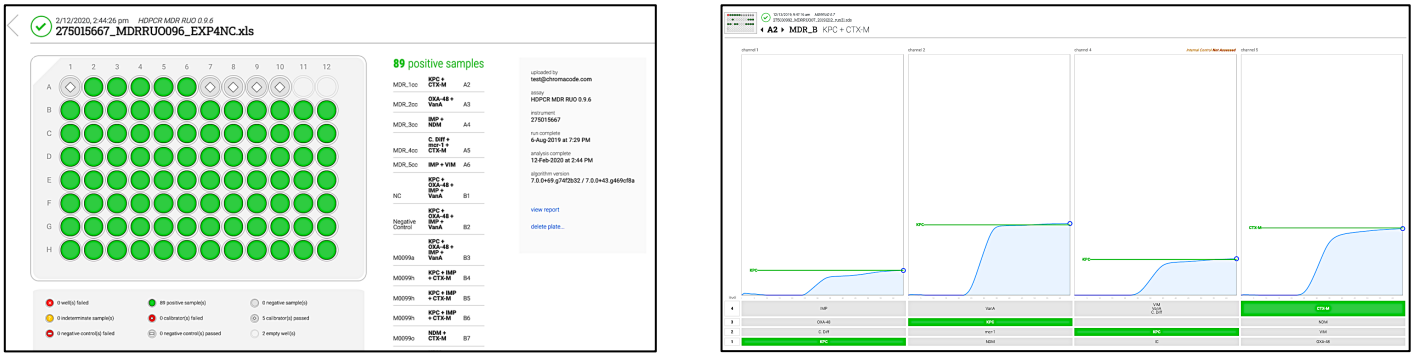

Figure 4: (Left) Plate Summary Page, which provides clear visualization of results across a 96-well plate and streamlined navigation to a well of interest; (Right) Well Summary Page provides the COVID+ test design beneath the four channels utilized in the COVID+ assay.

Discussion

Without clarity on the potential for co-infection of common respiratory pathogens with SARS-CoV-2 for the upcoming respiratory season, it will be important to have reliable tools that can minimally test for influenza, RSV, and SARS-CoV-2, enabling labs to study this phenomena. As currently designed, COVID+ would provide the following benefits to laboratories:

- ***Comprehensive multiplex test***. Simultaneous testing for influenza A, influenza B, RSV A&B, and SARS-CoV-2 in a single reaction from common upper respiratory sample types.
- ***High efficiency.*** Test for up to ~375 different samples in a single reaction with a 384-well plate and up to ~88 samples with a 96-well plate in under 2 hours.
- ***Leverages instruments already in labs.*** COVID+ is designed to be compatible with the most common automated nucleic extraction instruments and qPCR instruments.
- ***Streamlined data management with ChromaCode Cloud.*** Test result interpretation is extremely simple in the ChromaCode Cloud software, which can be easily integrated to push test results to data management software.

Additional studies with COVID+ still need to be performed to better understand the accuracy of the assay in samples with co-infections present at similar and different concentrations, as this is an area where molecular assays have historically struggled to perform with the same accuracy as single target detections.

Conclusions

ChromaCode's HDPCR Flu A / Flu B / RSV / SARS-CoV-2 (COVID+) assay will be able to provide laboratories and an accurate and efficient high-throughput PCR testing option for influenza, RSV, and SARS-CoV-2.

References:

1. Kim et al. Rates of Co-Infection Between SARS-CoV-2 and Other Respiratory Pathogens. *JAMA* Research Letter. https://jamanetwork.com/journals/jama/fullarticle/2764787. Accessed June 2, 2020.
2. Nowak et al. Co-infection in SARS-CoV-2 Infected Patients: Where are Influenza Virus and Rhinovirus/Enterovirus? *J Med Virol*. 2020 April 30. doi: 10.1002/jmv.25953.
3. Mengeghetti et al. What are the seasonal patterns of rhinoviral, coronaviral, enteroviral, and adenoviral upper respiratory tract infections (URIs)?. https://www.medscape.com/answers/302460-86798/what-are-the-seasonal-patterns-of-rhinoviral-coronaviral-enteroviral-and-adenoviral-upper-respiratory-tract-infections-uris. Accessed May 26, 2020.

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