

*Clinical and Analytical
Evaluation of the
Applied BioCode CoV-2
Flu Plus* Assay using
MagNA Pure 96 and
KingFisher Extraction
Systems*



12130 Mora Dr., Unit 2, Santa Fe Springs, CA 90670, USA



www.ApBioCode.com

Telephone: 1 (562) 777-9800 Fax: 1 (562) 777-9805

Clinical and Analytical Evaluation of the Applied BioCode CoV-2 Flu Plus Assay using MagNA Pure 96 and KingFisher Extraction Systems

Introduction

The morbidity and mortality of viral respiratory infections has become one of the greatest struggles facing healthcare providers, policy makers and patients today. Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is highly contagious respiratory syndrome responsible for over 500 million cases and 6.3 million deaths worldwide (1). The U.S., Australia, and Europe experienced unprecedented widespread outbreaks of Respiratory Syncytial Virus in 2021 and the threat of severe seasonal influenza and pandemic influenza requires constant vigilance (2).

With the overlapping symptoms of SARS-CoV2, Influenzas A & B and RSV, it is imperative to diagnose the true cause of these symptoms as rapidly and accurately as possible, which is why hospital labs have asked Applied BioCode to expand the use of the BioCode® CoV-2 Flu Plus Assay. The Centers for Disease Control and Prevention (CDC) strongly recommends that hospital labs order multiplex nucleic acid detection assay for both influenza A & B and SARS-CoV-2 to quickly determine etiology and to expedite the best possible treatment regimen (3). Having the correct assays and analyzers is vital to hospital labs to avoid bottlenecks and accurately detect viral respiratory infections. Rapid and accurate pathogen identification helps clinicians by providing the reliable data they need to make the best possible treatment decisions.

The BioCode® CoV-2 Flu Plus Assay is a multiplex molecular diagnostic test intended for the simultaneous qualitative detection and identification of SARS-CoV-2, Influenza A with subtypes (Seasonal H1, 2009 H1N1 & H3), Influenza B and RSV from a single nasopharyngeal swab specimen. The BioCode® CoV-2 Flu Plus was validated using with the NucliSENS easyMag (bioMérieux) and/or the MagNA Pure96 (Roche) nucleic acid extraction platforms (4).

This white paper demonstrates equitable results in the clinical performance of the BioCode CoV-2 Flu Plus Assay using either the MagNA Pure 96 or the KingFisher (ThermoFisher) extraction systems. This study used retrospective clinical patient samples to determine the Limit of Detection (LoD) with both, the MagNA Pure 96 and the KingFisher extraction platforms using quantified materials.

Background

The BioCode® CoV-2 Flu Plus Assay for use with the BioCode MDx-3000 system, which received Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration in December of 2021, is designed to assist clinicians in evaluating patients with clinical signs and symptoms for upper respiratory infections.

The test relies on the Applied BioCode proprietary Barcoded Magnetic Beads (BMB) technology for multiplex detection. The company has combined “digital barcodes” with molecular chemistry to create BMB technology. The BMBs, about the diameter of a human hair, are tagged with molecular probes, allowing the digital barcodes to be easily scanned. This system provides high accuracy with no ambiguity of biological targets.



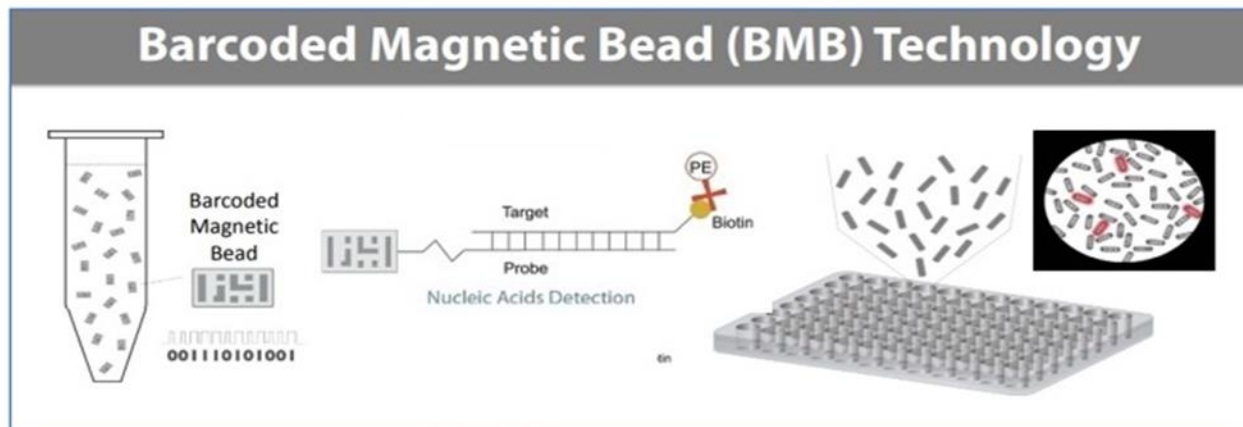


Figure 1: Barcoded Magnetic Beads (BMBs) are coupled to nucleic acid probes for capturing target nucleic acids in microtiter plates. For the BioCode® CoV-2 Flu Plus, biotinylated RT-PCR product is captured by target-specific probes coupled to BMBs, then labeled with SA-PE for detection.

The BioCode® CoV-2 Flu Plus Assay is run on the Applied BioCode's automated, high-throughput MDx-3000 Molecular System and provides scalable throughput, allowing clinical labs to run up to 188 sample per 8-hour shift.

BioCode® CoV-2 Flu Plus Assay builds on the existing portfolio of high-throughput syndromic assays from Applied BioCode including the FDA 510(k) cleared BioCode® Gastrointestinal Pathogen Panel (17 targets), FDA 510(k) cleared BioCode Respiratory Pathogen Panel (17 targets), and its stand-alone EUA authorized BioCode® SARS-CoV-2 Assay.

Methods

The BioCode® CoV-2 Flu Plus Assay is a molecular assay based on reverse transcription polymerase chain reaction (RT-PCR) and end-point detection of amplified DNA sequences with analyte-specific probes that are coupled to barcoded magnetic beads (BMB). The test is intended for the simultaneous qualitative detection and differentiation of RNA from:

- SARS-CoV-2
- Influenza A, including H1 pdm09, H1 seasonal, H3 subtypes
- Influenza B
- Respiratory syncytial virus (RSV)

According to the BioCode® CoV-2 Flu Plus Emergency Use Authorization, nucleic acids from Nasopharyngeal swab (NPS) specimens are extracted with the NucliSENS® easyMAG® (bioMérieux) or MagNA Pure 96 (Roche) automated systems. Once the PCR plate is set up and sealed, the rest of the operations are automated on the MDx-3000.



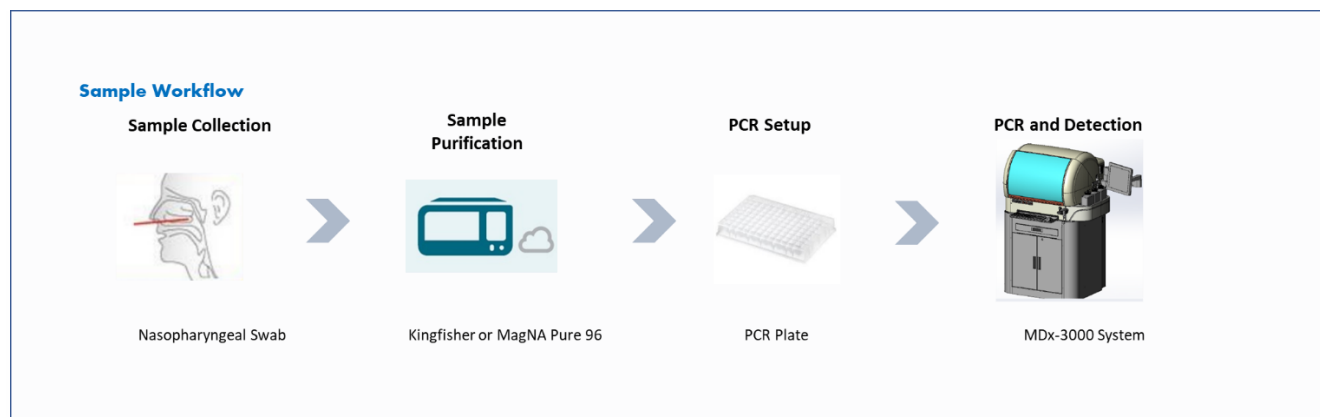


Figure 2: Sample workflow for the BioCode® MDx 3000 platform.

(ThermoFisher Scientific KingFisher nucleic acid extraction system is not approved for use with the BioCode® CoV-2 Flu Plus Assay, according to the Emergency Use Exemption issued by the FDA December 16, 2021.)

To demonstrate the performance and equivalency between two different extraction methods, the KingFisher Flex benchtop automated extraction instrument was evaluated as an alternative extraction method to MagNA Pure 96 by comparing results between the two systems using the BioCode CoV-2 Flu Plus Assay. Following extractions, the extracts from both MagNA Pure 96 and the KingFisher Flex were tested with the BioCode® MDx 3000 platform.

	MagNaPure 96	KingFisher Flex
 Sample type	UTM/VTM	UTM/VTM
 Sample input volume	0.2 mL	0.2 mL
 Elution volume	50 <u>uL</u>	50 <u>uL</u>
 Total processing time	50-60 min.	23 min.
 Hands-on-time	20-45 min.	30-60 min.
 Reaction (<u>rxn</u>)	576 <u>rxns</u>	1000 <u>rxns</u>
 Catalog number	6543588001	A48383R



Results

- 100 percent positive clinical agreement was achieved with both the KingFisher Flex and MagNA Pure 96 extraction systems for 30 Influenza A positive frozen NPS specimens.
- Of 43 Influenza B positive frozen NPS specimens, 42 were accurately identified by both KingFisher and MagNA Pure96. One specimen was identified as negative by both platforms.
- Of 30 RSV positive frozen NPS specimens, one internal control failed on MagNA Pure96, while one specimen was identified as negative by both platforms. Clinical agreement on 29 of the 30 was achieved by KingFisher and 28 of the 30 was achieved by MagNA Pure96.
- Of 60 SARS-CoV2 positive frozen nasal specimens, 59 were accurately identified by both KingFisher and MagNA Pure96. One specimen was identified as negative by both platforms.
- Of 32 SARS-CoV2 negative frozen NPS specimens, 32 were accurately identified by KingFisher while 30 were accurately identified with MagNA Pure96.

Sample Type	Number of Samples	Number of Concordant Samples		Concordance between MagNa Pure 96 and KingFisher Flex
		MagNa Pure 96	KingFisher	
Influenza A positive frozen NPS specimens	30	30	30	100%
Influenza B positive frozen NPS specimens	43	42	42	100%
RSV positive frozen NPS specimens	30	28	29	96.6%
SARS-CoV2 positive frozen nasal specimens	60	59	59	100%
SARS-CoV2 negative frozen NPS specimens	32	30	32	93.8%

Table 2: Concordance of 5 sample types between the MagNA Pure 96 and KingFisher nucleic acid extraction systems.



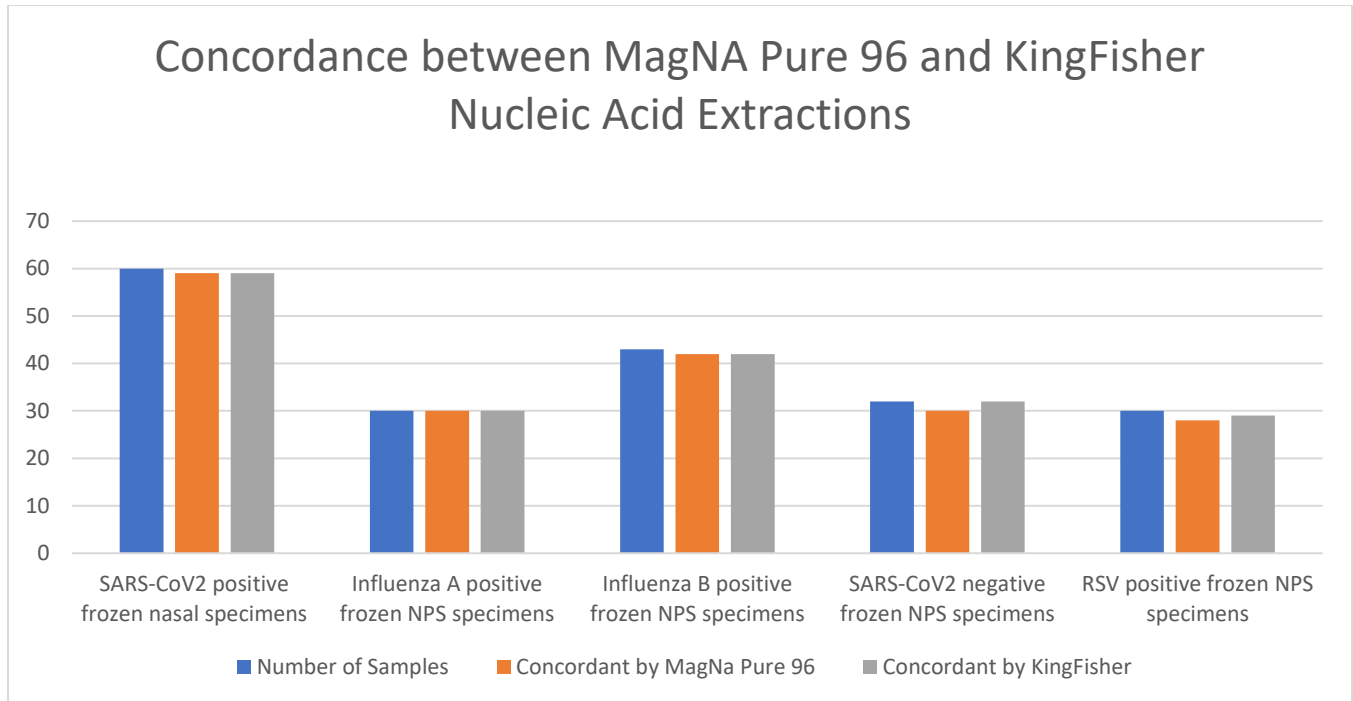


Figure 3: Concordance between MagNA Pure 96 and KingFisher nucleic acid extraction systems by sample type with an overall 97% concordance in positive identification

Comparison of the Two Nucleic Acid Extraction Systems

The two extraction systems share similar features including the sample loading specifications, processing limitations and automation. The extraction run time of MagNA Pure and the KingFisher system were 60 minutes and 30 minutes, respectively.

Nucleic Acid Extraction Performance: Sensitivity and LoD of the Extraction Systems for BioCode CoV-2 Flu Plus assay

The performances of the KingFisher Flex and the MagNA Pure 96 systems were comparable in efficiency, while KingFisher demonstrated slightly better concordance and LoDs. The LoDs for 6 virus strains were estimated with serial dilutions of each virus stock, and each dilution was tested in 4 replicates.

- SARS-CoV-2
- Influenza B (FluB)
- RSV
- Influenza A H1N1 (FluA H1 seasonal)
- Influenza A H1N1 Pandemic (FluA H1N1pdm09)
- Influenza A H3N2 (FluA H3N2)



Target	Species/Strain/Isolate	LoD Concentration (MagNA Pure 96)	LoD Concentration (KingFisher)
Influenza A H1N1	A/Brisbane/59/2007	2.6×10^{-1} TCID ₅₀ /mL	2.6×10^{-1} TCID ₅₀ /mL
Influenza A H1N1 pdm09	A/H1N1pdm/ Brisbane/02/2018	1.5×10^{-1} TCID ₅₀ /mL	0.5×10^{-1} TCID ₅₀ /mL
Influenza A H3N2	A/H3N2/ Hong Kong/2671/2019	4.5×10^0 TCID ₅₀ /mL	1.5×10^0 TCID ₅₀ /mL
Influenza B	Influenza B/ Brisbane/46/2015 (Victoria)	5.0×10^{-3} TCID ₅₀ /mL	5.0×10^{-3} TCID ₅₀ /mL
Respiratory Syncytial Virus	A Strain: 2/2015 Isolate #2	5.0×10^{-2} TCID ₅₀ /mL	5.0×10^{-2} TCID ₅₀ /mL
SARS-CoV-2	USA-WA1/2020 NCBI ACC #: MN985325	1.67×10^{-2} TCID ₅₀ /mL	1.67×10^{-2} TCID ₅₀ /mL

Table 3: The analytical sensitivity and limits of detection (LoD) were estimated with 6 virus strains with serial dilutions of each virus stock. Each dilution was tested in 4 replicates. Greater than 95% LoDs were achieved at the concentrations listed above.

Discussion

The COVID-19 pandemic demands rapid and accurate testing for large number of specimens from patients with symptoms of respiratory infection. To address the growing need for SARS-CoV2 detection and differentiation from other viral infections with overlapping symptoms, Applied BioCode launched the BioCode® CoV-2 Flu Plus on the MDx-3000 System.

For high-risk and immunocompromised patients for whom other viral and bacterial infections are possible, the BioCode® RPP test is available for the detection of the most common viruses and bacteria, including Influenza A and subtypes H1, H1N1 2009pdm and H3, Influenza B, Respiratory Syncytial Virus A/B, Parainfluenza virus types 1, 2, 3, and 4, Human Metapneumovirus A/B, Adenovirus, Rhinovirus/Enterovirus, Coronavirus (229E, OC43, HKU1, and NL63), *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Bordetella pertussis*.

Conclusion

To help alleviate the bottleneck in accurate and timely diagnosis of viral respiratory infection, PathAI Diagnostics tested the ThermoFisher Scientific's KingFisher nucleic acid extraction platform and determined it to be an acceptable extraction system for use with the BioCode COV-2 Flu Plus assay. In comparison to the MagNA Pure 96 extraction system, the KingFisher Flex performed well and provided equivalent results.



Acknowledgement:

This study was conducted at PathAI Diagnostics - <https://www.poplarhealthcare.com>
3495 Hacks Cross Road, Memphis, TN 38125

References

1. World Health Organization COVID-19 dashboard; <https://covid19.who.int/>
2. Branswell, H.; *STAT News*, May 25, 2022. "Viruses that were on hiatus during Covid are back — and behaving in unexpected ways"; <https://www.statnews.com/2022/05/25/viruses-that-were-on-hiatus-during-covid-are-back-and-behaving-in-unexpected-ways/>
3. Centers for Disease Control and Prevention; "Testing Guidance for Clinicians When SARS-CoV-2 and Influenza Viruses are Co-circulating" <https://www.cdc.gov/flu/professionals/diagnosis/testing-guidance-for-clinicians.htm>
4. Package Insert - BioCode® SARS-CoV-2 Flu Plus Assay kit - [BioCode® CoV-2 Flu Plus Assay Instructions for Use](#)

*This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, Influenza A (with H1 pdm09, H1 seasonal, H3 subtypes), Influenza B and/or Respiratory Syncytial Virus (RSV), not for any other viruses or pathogens; and The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

