510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number
K254139

B Applicant
Applied BioCode, Inc

C Proprietary and Established Names
BioCode Respiratory Pathogen Panel (RPP)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OCC, OZE, OEP, OEM, OOU, OTG, OZX, OZY, OZZ, NSU	II	21 CFR 866.3980 - Respiratory Viral Panel Multiplex Nucleic Acid Assay	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:
To obtain a substantial equivalence determination for the BioCode RPP for the detection of microbial and viral nucleic acids extracted from nasopharyngeal swab (NPS) samples for use on the Applied BioCode MDx 3000 instrument, using an alternate sample extraction system, the KingFisher Flex.

B Measurand:
Adenovirus, Human Metapneumovirus (hMPV) A/B, Influenza A (Flu A), Influenza A subtype H1 (Flu A/H1), Influenza A subtype H1 2009 Pandemic (Flu A/H1pdm09), Influenza A subtype H3 (Flu A/H3), Influenza B (Flu B), Coronavirus (229E, HKU1, OC43, and NL63), Parainfluenza virus 1 (PIV 1), Parainfluenza virus 2 (PIV 2), Parainfluenza virus 3 (PIV 3), Parainfluenza virus 4 (PIV 4), Human Rhinovirus/Enterovirus (HRV/HEV), Respiratory Syncytial Virus (RSV) A/B, *Bordetella pertussis*, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae* nucleic acid target sequences.

C Type of Test:
A multiplexed nucleic acid test intended for use with the BioCode MDx-3000 Instrument for the simultaneous qualitative *in vitro* detection and identification of multiple respiratory viral and

bacterial nucleic acids in nasopharyngeal swabs (NPS) collected in viral transport media (VTM) or universal transport media (UTM) and obtained from individuals suspected of respiratory tract infections.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The BioCode Respiratory Pathogen Panel (RPP) is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with BioCode MDx-3000 Instrument. The BioCode RPP is capable of the simultaneous detection and identification of nucleic acids from multiple viruses and bacteria extracted from nasopharyngeal swab (NPS) samples obtained from individuals with signs and/or symptoms of respiratory tract infection. The following pathogens and subtypes are identified using the BioCode RPP:

- Adenovirus
- Coronavirus (229E, OC43, HKU1, and NL63)
- Human Metapneumovirus A/B
- Influenza A, including subtypes H1, H1 2009 Pandemic, and H3
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus A/B
- Rhinovirus/Enterovirus
- *Bordetella pertussis*
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other organisms: the agent(s) detected by the BioCode RPP may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with a possible respiratory tract infection.

Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioCode RPP cannot differentiate them. A positive BioCode RPP Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is

required. The BioCode RPP detects Human Rhinovirus/Enterovirus with reduced sensitivity. If a more accurate HRV/EV result is required, it is recommended that specimens found to be negative for Human Rhinovirus/Enterovirus after examination using BioCode RPP be confirmed by an alternate method (e.g., FDA cleared molecular tests).

Performance characteristics for Influenza A were established when Influenza A H1 2009 Pandemic and A H3 were the predominant Influenza A viruses in circulation. Performance of detecting Influenza A may vary if other Influenza A strains are circulating, or a novel Influenza A virus emerges. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

C Special Conditions for Use Statement(s):
Rx – For Prescription Use Only

D Special Instrument Requirements:
BioCode MDx-3000 Instrument
bioMérieux NucliSENS easyMAG system
Roche MagNA Pure 96 system
Thermo Fisher KingFisher Flex automated systems

IV Device/System Characteristics:

A Device Description:
The BioCode Respiratory Pathogen Panel (RPP) is a respiratory pathogen multiplex nucleic acid test designed for use with the BioCode MDx-3000 system. The BioCode MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple viral and bacterial pathogens from a single nasopharyngeal swab specimen collected in VTM or UTM. Liquid specimens are processed, and nucleic acids extracted with the bioMérieux NucliSENS easyMAG, Roche MagNA Pure 96 or Thermo Fisher KingFisher Flex automated systems. Once the PCR plate is manually set up and sealed, all other operations are automated on the MDx-3000. The BioCode RPP simultaneously tests for 17 pathogens and/or subtypes from nasopharyngeal swab specimens collected in VTM or UTM and are listed below.

Viruses

- Adenovirus
- Coronavirus (229E, OC43, HKU1, and NL63)
- Human Metapneumovirus A/B
- Influenza A, including subtypes H1, H1 2009 Pandemic, and H3
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus A/B

- Rhinovirus/Enterovirus

Bacteria

- *Bordetella pertussis*
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

Results from the BioCode RPP test are available within about 5 hours.

B Principle of Operation:

The BioCode MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple respiratory viruses and bacteria from a single nasopharyngeal swab (NPS) specimen, in either VTM or UTM. Nucleic acids are extracted with the bioMérieux NucliSENS easyMAG, the Roche MagNA Pure 96 or the Thermo Fisher KingFisher Flex automated systems. Once the PCR plate is set up and sealed, all other operations are automated on the MDx-3000.

Nucleic Acid Extraction

Nucleic acids (both RNA and DNA) are captured by silica coated magnetic beads and eluted on the bioMérieux NucliSENS easyMAG, Roche MagNA Pure 96 or Thermo Fisher KingFisher Flex automated systems according to the manufacturer provided protocol.

Overview of a BioCode MDx-3000 Run

1. **Reverse Transcription and Multiplex PCR** – Since targets of the BioCode RPP include RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a freshly prepared reaction mixture for the RT step and subsequent thermal cycling for multiplex PCR to enrich the target nucleic acids present in the sample. One of the target-specific primers for each pathogen is biotinylated at the 5'-end to generate labeled PCR product for subsequent detection.

2. **Dispensing Barcoded Magnetic Bead (BMB)** – **Probe Mix** – Toward the end of PCR amplification, the robotic head dispenses BMB – Probe mix into the designated reaction wells of the capture plate using disposable pipette tips.

3. **PCR Product Transfer** – After PCR amplification is completed, the robotic head pierces the foil seal with disposable pipette tips and transfers PCR products into corresponding wells of the capture plate.

4. **Target Capture** – Amplified PCR products labeled with biotin are captured at a defined temperature by target-specific probes that are covalently coupled to designated BMBs. During this step, BMBs are kept in suspension by gentle agitation. Differentiation of captured targets is achieved by assigning a unique barcode pattern BMB for each pathogen and the internal control.

5. **Signal Generation** – After washing off unbound PCR products and unused primers, a

streptavidin-phycoerythrin (SA-PE) conjugate is automatically added to the reaction by the robot. High affinity binding between biotin and streptavidin ensures that captured PCR products with the biotin moiety are labeled with phycoerythrin in close proximity to the BMBs.

6. **Optical Detection** – Optical detection is performed for each reaction well of the capture plate, an optically clear, flat-bottom microtiter plate. After washing off unbound SA-PE, excitation of the fluorophore at the designated wavelength emits fluorescence signal from BMBs tagged with SA-PE conjugates. Each reaction well is imaged at a specific emission wavelength for fluorescent signal and under bright field for identifying the barcode patterns (decoding).

The BioCode MDx-3000 Software controls the operation of the instrument, collects and analyzes data, and automatically generates interpretation for test reports at the end of the run. Fluorescent signals from BMBs with the same barcode are sorted and calculated to generate a median fluorescence intensity (MFI) for each analyte. The presence or absence of a pathogen is determined relative to the validated assay cutoff by MFI. The software also analyzes the results of external and internal controls to validate the run and individual specimen results for reporting.

C Instrument Description Information:

1. Instrument Name:
   BioCode MDx-3000 Instrument

2. Specimen Identification:
   Specimen identity is provided by barcoded magnetic beads (BMB).

3. Specimen Sampling and Handling:
   After nucleic acid extraction with the bioMérieux NucliSENS easyMAG, the Roche MagNA Pure 96 or the Thermo Fisher KingFisher Flex automated systems and manually loading samples into a 96-well formatted plate, the BioCode MDx 3000 processes all RT-PCR, target capture, signal generation, and optical detection steps automatically.

4. Calibration:
   Optical calibration of the BioCode MDx 3000 is performed twice a year by Applied BioCode. No calibration kit is available.

5. Quality Control:
   Each laboratory is expected to establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations, and good laboratory practices. The BioCode RPP uses an internal control (bacteriophage MS2) which is added to each sample prior to extraction. The internal control monitors the efficiency of the extraction, reverse transcription, amplification, and detection stages of the assay. Positive results may be reported in the absence of RNA IC detection. The BioCode RPP software will suppress negative results for any wells with invalid RNA IC results.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioCode Respiratory Pathogen Panel (RPP)

B Predicate 510(k) Number(s):
K192485

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K254139**	**K192485 (Predicate)**
Device Trade Name	BioCode Respiratory Pathogen Panel (RPP)	BioCode Respiratory Pathogen Panel (RPP)
Common Name	Respiratory Virus Panel Nucleic Acid Assay System	Respiratory Virus Panel Nucleic Acid Assay System
Regulation	21CFR 866.3980	21CFR 866.3980
Product Code	OCC	OCC
Device Class	II	II
General Device Characteristic Similarities		
Intended Use	The BioCode Respiratory Pathogen Panel (RPP) is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BioCode MDx-3000 Instrument. The BioCode RPP is capable of the simultaneous detection and identification of nucleic acids from multiple viruses and bacteria extracted from nasopharyngeal swab (NPS) samples obtained from individuals with signs and/or symptoms of respiratory tract infection. The following pathogens and subtypes are identified using the BioCode RPP: • Adenovirus • Coronavirus (229E, OC43, HKU1, and NL63) • Human Metapneumovirus A/B • Influenza A, including subtypes H1, H1 2009 Pandemic, and H3 • Influenza B • Parainfluenza 1 • Parainfluenza 2	Same

	• Parainfluenza 3 • Parainfluenza 4 • Respiratory Syncytial Virus A/B • Rhinovirus/Enterovirus • *Bordetella pertussis* • *Chlamydia pneumoniae* • *Mycoplasma pneumoniae* The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Positive results do not rule out coinfection with other organisms: the agent(s) detected by the BioCode RPP may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with a possible respiratory tract infection. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioCode RPP cannot differentiate them. A positive BioCode RPP Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is required.	

	The BioCode RPP detects Human Rhinovirus / Enterovirus with reduced sensitivity. If a more accurate HRV/EV result is required, it is recommended that specimens found to be negative for Human Rhinovirus / Enterovirus after examination using BioCode RPP be confirmed by an alternate method (e.g., FDA cleared molecular tests). Performance characteristics for Influenza A were established when Influenza A H1 2009 Pandemic and A H3 were the predominant Influenza A viruses in circulation. Performance of detecting Influenza A may vary if other Influenza A strains are circulating, or a novel Influenza A virus emerges. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	
Specimen Types	NPS (in VTM or UTM)	Same
Pathogens Detected	Adenovirus, Coronavirus (229E, HKU1, NL63, and OC43), Human Metapneumovirus A/B, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 Pandemic, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2,	Same

	Parainfluenza Virus 3, Parainfluenza Virus 4, Rhinovirus/Enterovirus, Respiratory Syncytial Virus A/B, *Bordetella pertussis*, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*	
Analyte	RNA/DNA	Same
Instrumentation	Nucleic Acid Purification System BioCode MDx-3000	Same
Time to Result	About 5.0 hours	Same
Test Interpretation	Automated test interpretation and report generation	Same
Controls	An RNA Internal Control is added to each sample during extraction. External Positive and Negative Controls are externally sourced.	Same
Methodology	Multiplex RT-PCR and probe hybridization to biotinylated PCR product(s) followed by fluorescence detection and decoding of barcoded magnetic beads (BMB) that are coupled to target-specific probes	Same
CLIA Complexity	High	Same
Sample Extraction	easyMAG, Roche MagNA Pure 96, KingFisher Flex	easyMAG, Roche MagNA Pure 96
Internal Control Cut-off Values	13,000 MFI	8,000 MFI

VI Standards/Guidance Documents Referenced:

- FDA guidance document issued on August 27, 2014, titled *"Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices"*
- FDA guidance document issued on October 9, 2009, titled "*Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay"*
- FDA guidance document issued on October 9, 2009, titled "*Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Assays"*
- FDA guidance document issued on October 9, 2009, titled *"Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays"*
- FDA guidance document issued on July 15, 2011, titled *"Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses"*
- FDA guidance document issued on April 25, 2005, titled *"Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable"*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
A reproducibility study was conducted to assess the reproducibility of BioCode RPP with nucleic acid extraction performed using the KingFisher Flex system.

The reproducibility panel includes eight contrived samples composed of combinations of twelve representative targets (nine viral and three bacterial) at 1.5x Limit of Detection (LoD) (Low) and 3x LoD (Medium) spiked into pooled negative synthetic nasopharyngeal swab (sNPS) matrix, and one negative control sample (**Table 1**). Synthetic NPS matrix (sNPS) was previously determined to be equivalent to NPS matrix and was used for the LoD and reproducibility studies for the original 510(k) submission of BioCode RPP (K192485).

One lot of BioCode RPP was tested by three operators on five non-consecutive days. Each day the samples were extracted in triplicate on KingFisher Flex extraction instruments by each operator and assayed using BioCode RPP for a total of 90 extraction/PCR replicates per panel member.

Table 1. Reproducibility Panel – Sample Composition

Medium (3x LoD)	Medium (3x LoD)	Low (1.5x LoD)	Low (1.5x LoD)	Sample Name
Human Rhinovirus/ Enterovirus	Parainfluenza Virus 2	N/A	N/A	RP1a
N/A	N/A	Human Rhinovirus/Enterovirus	Parainfluenza Virus 2	RP1b
Human Metapneumovirus (hMPV)	*Bordetella pertussis*	N/A	N/A	RP2a
N/A	N/A	Human Metapneumovirus (hMPV)	*Bordetella pertussis*	RP2b
Influenza B	Coronavirus NL63	*Chlamydia pneumoniae*	Parainfluenza Virus 3	RP3
Chlamydia pneumoniae	Parainfluenza Virus 3	Influenza B	Coronavirus NL63	RP4
Influenza A H3N2	*Mycoplasma pneumoniae*	Respiratory Syncytial Virus (RSV)	Adenovirus C (ADV)	RP5
Respiratory Syncytial Virus (RSV)	Adenovirus C (ADV)	Influenza A H3N2	*Mycoplasma pneumoniae*	RP6
Negative matrix (synthetic NPS)				RP7

All targets tested at 3x LoD had 90/90 (100%) agreement with expected results except for Human Metapneumovirus and Bordetella pertussis (RP2a) which had 89/90 (98.9%) agreement with expected results. Reproducibility results are summarized in **Table 2**.

Table 2. Reproducibility Results

Analyte	Concentration Tested	Expected Result	Agreement with Expected Results
			KingFisher Flex Extraction (95% CI)
Viruses			

Adenovirus	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Coronavirus NL63	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Coronavirus HKU	None (no analyte)	Not Detected	810/810 / **100%** / (99.6% - 100%)
Coronavirus OC43	None (no analyte)	Not Detected	810/810 / **100%** / (99.6% - 100%)
Coronavirus 229E	None (no analyte)	Not Detected	809/810 / **99.9%** / (99.3% - 100%)
Human Metapneumovirus	3x LoD	Detected	89/90* / **98.9%** / (94.0%-99.8%)
	1.5x LoD	Detected	88/90 / **97.8%** / (92.3%-99.4%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Human Rhinovirus/ Enterovirus	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (89.1% - 98.3%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Influenza A/H3	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Influenza A/H1pdm09	None (no analyte)	Not Detected	810/810 / **100%** / (99.6% - 100%)
Influenza A/H1	None (no analyte)	Not Detected	810/810 / **100%** / (99.6% - 100%)
Influenza B	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Parainfluenza Virus 1	None (no analyte)	Not Detected	810/810 / **100%** / (99.6% - 100%)
Parainfluenza Virus 2	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	88/90 / **97.8%** / (92.3% - 99.4%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Parainfluenza Virus 3	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Parainfluenza Virus 4	None (no analyte)	Not Detected	810/810 / **100%** / (99.6% - 100%)
Respiratory Syncytial Virus	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	629/630 / **99.8%** / (99.1% - 100%)
Bacteria			
Mycoplasma pneumoniae	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)
Bordetella pertussis	3x LoD	Detected	89/90* / **98.9%** / (94.0% - 100%)
	1.5x LoD	Detected	88/90 / **97.8%** / (92.3% - 99.4%)
	None (no analyte)	Not Detected	629/630 / **99.8%** / (99.1% - 100%)
Chlamydia pneumoniae	3x LoD	Detected	90/90 / **100%** / (95.9% - 100%)
	1.5x LoD	Detected	89/90 / **98.9%** / (94.0% - 99.8%)
	None (no analyte)	Not Detected	630/630 / **100%** / (99.4% - 100%)

* Missed replicate due to PCR set up error detected in (3/3) repeat replicates on the same instrument. It was not due to KFF extraction.

Descriptive statistical calculations of Median Fluorescence Intensity (MFI) (average, standard deviation and percent coefficient of variation) between runs and between days, are presented in **Table 3**.

Table 3. Median Fluorescence Intensity (MFI) Summary Statistics Between Runs and Days

Target	Concentration	Average ± Standard Deviation (%CV)	
		Between Runs	Between Days
Adenovirus 1	3x LoD	9884 ± 2049 (21%)	9878 ± 892 (9%)
	1.5x LoD	9362 ± 2204 (24%)	9261 ± 659 (7%)
Adenovirus 2	3x LoD	14595 ± 2668 (18%)	14633 ± 937 (6%)
	1.5x LoD	13813 ± 2887 (21%)	13694 ± 770 (6%)
Coronavirus NL63	3x LoD	31076 ± 2403 (8%)	30814 ± 830 (3%)
	1.5x LoD	27451 ± 1648 (6%)	27369 ± 629 (2%)
Human Metapneumovirus 1	3x LoD	34769 ± 2343 (7%)	34552 ± 632 (2%)
	1.5x LoD	32704 ± 2984 (9%)	32761 ± 1189 (4%)
Human Metapneumovirus 2	3x LoD	27057 ± 3351 (12%)	27047 ± 1483 (5%)
	1.5x LoD	24061 ± 2560 (11%)	24435 ± 1320 (5%)
Human Rhinovirus	3x LoD	26049 ± 3607 (14%)	26356 ± 678 (3%)
	1.5x LoD	20419 ± 3665 (18%)	20713 ± 933 (5%)
Influenza A/H3	3x LoD	37536 ± 3920 (10%)	37233 ± 1124 (3%)
	1.5x LoD	33004 ± 3120 (9%)	33045 ± 303 (1%)
Influenza B1	3x LoD	7590 ± 1495 (20%)	7685 ± 675 (9%)
	1.5x LoD	8453 ± 1273 (15%)	8352 ± 1059 (13%)
Influenza B2	3x LoD	28916 ± 4178 (14%)	28880 ± 1641 (6%)
	1.5x LoD	24176 ± 4530 (19%)	24800 ± 1468 (6%)
Parainfluenza Virus 2	3x LoD	22276 ± 2278 (10%)	22081 ± 860 (4%)
	1.5x LoD	17004 ± 2547 (15%)	16910 ± 1476 (9%)
Parainfluenza Virus 3	3x LoD	25157 ± 2082 (8%)	25087 ± 287 (1%)
	1.5x LoD	23631 ± 2247 (10%)	23624 ± 780 (3%)
Respiratory Syncytial Virus	3x LoD	36077 ± 4711 (13%)	36023 ± 1561 (4%)
	1.5x LoD	38821 ± 3397 (9%)	38874 ± 1477 (4%)
Mycoplasma pneumoniae	3x LoD	44239 ± 4679 (11%)	44153 ± 2598 (6%)
	1.5x LoD	39045 ± 7167 (18%)	38573 ± 3577 (9%)
Bordetella pertussis	3x LoD	14422 ± 4935 (34%)	14666 ± 1934 (13%)
	1.5x LoD	11114 ± 3865 (35%)	11519 ± 1510 (13%)
Chlamydia pneumoniae	3x LoD	26886 ± 4646 (17%)	26443 ± 2692 (10%)
	1.5x LoD	22558 ± 4124 (18%)	22400 ± 1425 (6%)

2. Linearity:
   Not applicable, BioCode RPP is a qualitative assay

3. Analytical Specificity/Interference:
   Inclusivity, microbial interference, cross-reactivity and potential interfering substance of BioCode RPP was evaluated in the original 510(k) Premarket Notification (K192485). No additional testing was conducted.

   Competitive Inhibition

   An analytical study was performed to evaluate potential competitive inhibition for Adenovirus species C Serotype 2 (AdVC2) and *Bordetella pertussis* (Bp) on the BioCode RPP when using the KFF extraction method.

Competitive inhibition of AdVC2 and Bp were evaluated by spiking potential inhibitory analytes in competitive inhibition samples at high concentration ($\geq 10^5$ for viruses) and the two targets at low concentration (3x LoD).

Summary results of this study are presented in **Table 4** below. No inhibition was observed at the concentrations tested in this study.

Table 4. Competitive Inhibition Study Results

Panel Designation	Viral/Bacterial Strain	Source	Level	Titer Tested	Detected (n of 3)	Pass/Fail
Competitive Inhibition Sample 1	Respiratory Syncytial Virus Type A	Zeptometrix 0810040ACF	High	$1x10^5$ $TCID_{50}$/mL	3/3	Pass
	Influenza A H3N2 A/Alice	ATCC VR-776	Low	81 $CEID_{50}$/mL	3/3	
	Adenovirus species C Serotype 2	ATCC VR-846	Low	54 $TCID_{50}$/mL	3/3	
Competitive Inhibition Sample 2	Influenza A H3N2 A/Alice	ATCC VR-776	High	$1x10^5$ $CEID_{50}$/mL	3/3	Pass
	Adenovirus species C Serotype 2	ATCC VR-846	Low	54 $TCID_{50}$/mL	3/3	
	Respiratory Syncytial Virus Type A	Zeptometrix 0810040ACF	Low	0.99 $TCID_{50}$/mL	3/3	
Competitive Inhibition Sample 3	Coronavirus OC43	Zeptometrix 0810024CF	High	$1x10^5$ $TCID_{50}$/mL	3/3	Pass
	Human Metapneumovirus	Zeptometrix 0810161CF	Low	5 $TCID_{50}$/mL	3/3	
	Bordetella pertussis	Zeptometrix 081459	Low	810 CFU/mL	3/3	
Competitive Inhibition Sample 4	Human Metapneumovirus	Zeptometrix 0810161CF	High	$1x10^5$ $TCID_{50}$/mL	3/3	Pass
	Bordetella pertussis	Zeptometrix 081459	Low	810 CFU/mL	3/3	
	Coronavirus OC43	Zeptometrix 0810024CF	Low	0.27 $TCID_{50}$/mL	3/3	

4. Detection Limit and Assay Reportable Range:

The LoD of BioCode RPP was evaluated with both KFF and MagNA Pure 96 (MP96) extraction systems using simulated NPS matrix samples spiked with target analytes. An estimated LoD for each analyte was determined by testing serial dilutions of contrived samples. Four extraction replicates per dilution on each extraction system, KFF and MP96, were tested using the BioCode RPP.

The LoD for each analyte was confirmed by testing 20 replicates extracted with each extraction system. LoD for each isolate was defined as the lowest concentration with ≥95% detection of 20 replicates (at least 19 out of 20 replicates). Summary results of the LoD confirmation study are presented in **Table 5**.

Table 5. Limit of Detection for the KingFisher Extraction System – Comparison with MagNA Pure 96

Organism	Strain	Source	MagNA Pure 96	KingFisher Flex

			LoD	Detection (n/20)	LoD	Detection (n/20)
Influenza A H1N1	A/New Caledonia/20/99	Zeptometrix 0810036CF	$1.67x10^{0}$ TCID50/mL	20/20	$5.00x10^{0}$ TCID50/mL	20/20
Influenza A H1N1	A/NWS/33	ATCC VR-219	$2.70x10^{1}$ TCID50/mL	20/20	$2.70x10^{1}$ TCID50/mL	20/20
Influenza A H1N1 pdm09	H1N1 California/07/09	Zeptometrix 0810165CF	$4.00x10^{-1}$ TCID50/mL	20/20	$4.00x10^{-1}$ TCID50/mL	20/20
Influenza A H3N2	A/Wisconsin/67/05	Zeptometrix 0810252CF	$4.33x10^{-1}$ TCID50/mL	19/20	$1.30x10^{0}$ TCID50/mL	20/20
Influenza A H3N2	A/Alice	ATCC VR-776	$9.00x10^{0}$ CEID50/.2mL	19/20	$2.70x10^{1}$ CEID50/mL	20/20
Influenza B	Flu B/Florida/4/2006 (Yamagata)	Zeptometrix 0810255CF	$1.00x10^{-2}$ TCID50/mL	20/20	$6.00x10^{-2}$ TCID50/mL	20/20
Influenza B	B/Hong Kong/5/1972 (Victoria)	ATCC VR-823	$1.80x10^{0}$ TCID50/mL	20/20	$5.40x10^{0}$ TCID50/mL	19/20
Respiratory Syncytial Virus	Type A	Zeptometrix 0810040ACF	$1.10x10^{-1}$ TCID50/mL	20/20	$3.30x10^{-1}$ TCID50/mL	20/20
Human Metapneumovirus	16; Type A1 IA10-2003	Zeptometrix 0810161CF	$5.56x10^{-1}$ TCID50/mL	20/20	$1.67x10^{0}$ TCID50/mL	20/20
Parainfluenza Virus 1	[C-35/Washington DC/1957]	Zeptometrix 0810014CF	$9.00x10^{0}$ TCID50/mL	20/20	$9.00x10^{0}$ TCID50/mL	20/20
Parainfluenza Virus 2	[Greer/Ohio/1955]	ATCC VR-92	$1.80x10^{0}$ TCID50/mL	20/20	$5.40x10^{0}$ TCID50/mL	19/20
Parainfluenza Virus 3	N/A	Zeptometrix 0810016CF	$5.00x10^{0}$ TCID50/mL	19/20	$1.50x10^{1}$ TCID50/mL	20/20
Parainfluenza Virus 4	type 4a	Zeptometrix 0810060CF	$2.70x10^{1}$ TCID50/mL	20/20	$8.10x10^{1}$ TCID50/mL	20/20
Adenovirus	Species B Serotype 7A	Zeptometrix 0810021CF	$1.20x10^{0}$ TCID50/mL	20/20	$3.60x10^{0}$ TCID50/mL	20/20
Adenovirus	Species C Serotype 2	ATCC VR-846	$2.00x10^{0}$ TCID50/mL	19/20	$1.80x10^{1}$ TCID50/mL	20/20
Adenovirus	Species E Serotype 4	Zeptometrix 0810070CF	$4.00x10^{-2}$ TCID50/mL	20/20	$1.33x10^{-1}$ TCID50/mL	19/20
Coronavirus 229E	229E	Zeptometrix 0810229CF	$6.00x10^{-1}$ TCID50/mL	20/20	$1.80x10^{0}$ TCID50/mL	20/20
Coronavirus HKU1	HKU1[a]	Clinical Sample	$1.50x10^{5}$ copies/mL	20/20	$1.50x10^{5}$ copies/mL	19/20
Coronavirus NL63	NL63	Zeptometrix 0810228CF	$1.33x10^{-2}$ TCID50/mL	20/20	$4.00x10^{-2}$ TCID50/mL	20/20
Coronavirus OC43	OC43	Zeptometrix 0810024CF	$3.00x10^{-2}$ TCID50/mL	20/20	$9.00x10^{-2}$ TCID50/mL	20/20
Human Rhinovirus/Enterovirus	Rhinovirus Type A1	Zeptometrix 0810012CFN	$4.00x10^{-1}$ TCID50/mL	20/20	$4.00x10^{-1}$ TCID50/mL	20/20
Enterovirus	Enterovirus D68	Zeptometrix 0810300CF	$9.00x10^{0}$ CFU/mL	19/20	$9.00x10^{0}$ CFU/mL	19/20
Bordetella pertussis	A639	Zeptometrix 0801459	$1.50x10^{1}$ CFU/mL	20/20	$2.70x10^{2}$ CFU/mL	20/20
Chlamydia pneumoniae	TW-183 (AR39)	ATCC 53592	$3.30x10^{1}$ IFU/mL	20/20	$3.30x10^{1}$ IFU/mL	20/20
Mycoplasma pneumoniae	M129	Zeptometrix 0801579	$1.50x10^{1}$ CCU/mL	20/20	$4.50x10^{1}$ CCU/mL	20/20

[a] Coronavirus HKU1 is not available as a titered stock. A positive patient sample, quantified in copies/mL using a PCR standard curve created using quantified *in vitro* RNA, was used.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

No modifications were made to BioCode RPP or MDx 3000 instrument system. Please refer to the published decision summary for the original 510(k) submission for additional information (K192485).

6. Assay Cut-Off:
MFI cutoff values were not modified for analyte detection, please refer to the published decision summary for the original 510(k) submission for additional information (K192485). The MFI cutoff value for the Internal Control (IC) RNA (bacteriophage MS2) was increased from 8,000 to 13,000 MFI.

B Comparison Studies:

1. Method Comparison with Predicate Device:
To support the performance of the BioCode RPP when used with the KFF extraction system, a total of 735 (629 positive and 106 negative) archived NPS clinical samples collected from patients across five geographically diverse investigational sites were evaluated with both the KFF extraction system and previously cleared methods employing the MP96 extraction system. These remnant samples were used to support the original 510(k) clearance of the BioCode RPP device.

Ten (10) deidentified, frozen remnant Bp positive samples were used for Bp method comparison testing. In addition, a total of 300 samples were contrived at 2x LoD and 4x LoD (25 each) and tested to determine the performance characteristics for *Bordetella pertussis, Chlamydia pneumonia, Mycoplasma pneumonia*, Influenza A H1N1, Parainfluenza 1 and Parainfluenza 4. The demographics of the enrolled subjects in the clinical study are shown in **Table 6** and the demographics of the Bp positive samples are shown in **Table 9**.

Table 6. Demographic data for Archived Samples

Archived Samples	
Total Specimen Count	735
Sex	
Male	374/735 (50.9%)
Female	361/735 (49.1%)
Age	
≤ 5 yrs	368/735 (50.1%)
6-21 yrs	197/735 (26.8%)
22-59 yrs	89/735 (12.1%)
60+ yrs	81/735 (11.0%)

Positive percent agreement (PPA) was calculated as TP/(TP + FN)(TP = true positive or positive by both the MP96 and KFF; FN = false negative or negative by the KFF only). Negative percent agreement was calculated as TN/(TN + FP)(TN= true negative or negative by the MP96 and KFF; FP = false positive or positive by KFF only). Performance of BioCode RPP using KFF extraction method is shown in **Tables 7, 8 and 10**.

Table 7. Results from archived samples tested with BioCode RPP using KFF extraction compared to MP96 extraction

Target	**Positive Agreement**		**Negative Agreement**	
	PPA %	**95% CI**	**NPA (%)**	**95% CI**
Influenza A	110/110 (**100%**)	96.6-100%	624/625 (**99.8%**)	99.1-100%
Influenza A (H1N1 seasonal)	N/A	N/A	735/735 (**100%**)	99.5-100%

Influenza A (H1N1 pandemic 09)	30/30 (**100%**)	88.6-100%	705/705 (**100%**)	99.5-100%
Influenza A (H3)	75/76 (**98.7%**)	92.9-99.8%	658/659 (**99.8%**)	99.1-100%
Influenza B	35/37 (**94.6%**)	82.3-98.5%	698/698 (**100%**)	99.5-100%
Respiratory Syncytial Virus (RSV)	104/114 (**91.2%**)	84.6-95.2%	620/621 (**99.8%**)	99.1-100%
Human Metapneumovirus	63/65 (**96.9%**)	89.5-99.2%	670/670 (**100%**)	99.4-100%
Parainfluenza virus 1	N/A	N/A	734/734 (**100.0%**)	99.5-100%
Parainfluenza virus 2	31/31 (**100%**)	89.0-100%	703/704 (**99.9%**)	99.2-100%
Parainfluenza virus 3	64/69 (**92.8%**)	84.1-96.9%	663/666 (**99.5%**)	98.7-99.8%
Parainfluenza virus 4	N/A	N/A	733/734 (**99.9%**)	99.2-100%
Adenovirus	88/97 (**90.7%**)	83.3-95.0%	632/638 (**99.1%**)	98.0-99.6%
Coronavirus (229E, HKU1, NL63, OC43)	59/61 (**96.7%**)	88.8-99.1%	672/674 (**99.7%**)	98.9-99.9%
Rhinovirus/Enterovirus	129/135 (**95.6%**)	90.6-97.9%	595/600 (**99.2%**)	98.1-99.6%
Bordetella pertussis	N/A	N/A	727/729 (**99.7%**)	99.0-99.9%
Mycoplasma pneumoniae	N/A	N/A	733/734 (**99.9%**)	99.2-100%
Chlamydia pneumoniae	N/A	N/A	735/735 (**100%**)	99.5-100%
Combined Targets	788/825 (**95.5%**)	93.9-96.7%	11,637/11,661 (**99.8%**)	99.7-99.9%

Table 8. Results from contrived samples tested by BioCode RPP using the KFF extraction system compared to MP96

Target	Strain/ Isolate	Fold LoD	Concentration	PPA (%)	95% CI	NPA (%)	95 CI
Bordetella pertussis	A639	2x	$5.40x10^2$ CFU/mL	22/23[a] (95.7%)	79.0%-99.2%	249/251[b] (99.2%)	97.1%-99.8%
		4x	$1.08x10^3$ CFU/mL	25/25 (100%)	86.7%-100%		
			Combined	47/49 (95.9%)	86.3%-98.9%		
Chlamydia pneumoniae	TW-183 (AR39)	2x	$6.67x10^1$ IFU/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
		4x	$1.33x10^2$ IFU/mL	25/25 (100%)			
			Combined	50/50 (100%)	92.9%-100%		
	A/New	2x	$1.00x10^1$ $TCID_{50}$/mL	25/25 (100%)	86.7%-100%		98.5%-

Influenza A H1	Caledonia/20/99	4x	$2.00x10^1$ $TCID_{50}$/mL	25/25 (100%)		250/250 (100%)	100%
			Combined	50/50 (100%)	92.9%-100%		
Mycoplasma pneumoniae	M129	2x	$9.00x10^1$ CCU/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
		4x	$1.80x10^2$ CCU/mL	25/25 (100%)			
			Combined	50/50 (100%)	92.9%-100%		
Parainfluenza Virus 1	C-35/ Washington DC/ 1957	2x	$1.80x10^1$ $TCID_{50}$/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
		4x	$3.60x10^1$ $TCID_{50}$/mL	25/25 (100%)			
			Combined	50/50 (100%)	92.9%-100%		
Parainfluenza Virus 4	Type 4a	2x	$1.62x10^2$ $TCID_{50}$/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
		4x	$3.24x10^2$ $TCID_{50}$/mL	25/25 (100%)			
			Combined	50/50 (100%)	92.9%-100%		

[a]25 samples contrived with BP at 2x LoD were tested. BP was detected in 23/25 from the MP96 extracts and 24/25 from the KFF extracts.
[b] *Bordetella pertussis* was detected in one of the 4x LoD PIV4 MP96 extracted contrived samples

Table 9. Demographic data for *Bordetella pertussis* samples

Remnant Samples	
Total Specimen Count	10
Sex	
Male	3/10 (30%)
Female	7/10 (70%)
Age Category	
≤ 5 yrs	3/10 (30%)
6-21 yrs	6/10 (60%)
22-59 yrs	1/10 (10%)
60 + yrs	0/10 (0%)

Table 10. Method Comparison Results: RPP Bp (MP96 Extraction vs KFF Extraction)

Target	Positive Agreement (MP96 extraction vs KFF extraction)	
	PPA % [a]	95% CI
Bordetella pertussis	10/10 (100%)	74.1-100%

[a] Positive percent agreement (PPA; [true positive/true positive + false negative] x 100)

2. Matrix Comparison:
   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:
   Not applicable.

2. Clinical Specificity:
   Not applicable.

3. Clinical Cut-Off
   Not applicable.

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   None

D Expected Values/Reference Range:
Refer to the published decision summary for the original clearance in K192485.

E Other Supportive Instrument Performance Characteristics Data:
Refer to the published decision summary for the original clearance in K192485.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.