May 1, 2026

Applied BioCode, Inc.
Alex Chang
Regulatory Consultant
12130 Mora Dr. Unit 2,
Santa Fe Springs, CA 90670 USA

Re: K254139
Trade/Device Name: BioCode Respiratory Pathogen Panel (RPP)
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OCC, NSU , OEM , OEP , OOU , OTG , OZE , OZX , OZY , OZZ
Dated: December 19, 2025
Received: December 22, 2025

Dear Alex Chang:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality Management System Regulation (QMSR) (21 CFR Part 820), which includes, but is not limited to, ISO 13485 clause 7.3 (Design controls), ISO 13485 clause 8.3 (Nonconforming product), and ISO 13485 clause 8.5.2 (Corrective action), and ISO 13485 clause 8.5.3 (Preventative action). Please note that regardless of whether a change requires premarket review, the QMSR requires device manufacturers to review and approve changes to device design and production (ISO 13485 clause 7.3 and ISO 13485 clause 7.5) and document changes and approvals in the Medical Device File (ISO 13485 clause 4.2.3).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the Quality Management System Regulation (QMSR) (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

JOSEPH BRIGGS -S

Joseph Briggs, Ph.D.
Deputy Division Directer
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K254139

Device Name
BioCode Respiratory Pathogen Panel (RPP)

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Prepared in accordance with the requirements of 21 CFR 807.92.

I. Submitter Information [807.92(a)(1)]

Submitter/ Applicant	Applied BioCode®, Inc. 12130 Mora Drive Santa Fe Springs, CA 90670 Submitter Contact: Alex Chang Phone: +1 (408) 839-5826 Email: achang@apbiocode.com
Date Prepared	December 19, 2025

II. Device Information [807.92(a)(2)]

Trade Name	BioCode Respiratory Pathogen Panel (RPP)
Classification Name	Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulation	21 CFR § 866.3980

III. Predicate Device [807.92(a)(3)]

K192485	BioCode Respiratory Pathogen Panel (RPP)

IV. Device Description [807.92(a)(4)]

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. The pathogens and/or subtypes are:

- 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*

Results from the BioCode RPP test are available within about 5 hours. The following targets/ internal controls are included in the BioCode RPP.

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

V. Intended Use/ Indications for Use [807.92(a)(5)]

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 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.

VI. Device Comparison [807.92(a)(6)]:

Comparison of the Applied BioCode RPP with the Predicate Device

Characteristic	Proposed Device	Predicate
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
510(k) No.	N/A	K192485
Regulation	21CFR 866.3980	21CFR 866.3980
Product Code	OCC	OCC
Device Class	II	II
Similarities		
Intended Use	The BioCode® 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	Same

Characteristic	Proposed Device	Predicate
	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.	

Characteristic	Proposed Device	Predicate
	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	Same

Characteristic	Proposed Device	Predicate
	subtype H3, Influenza A subtype 2009 Pandemic, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, 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	
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
Differences		
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

VII. Summary of Performance Data [807.92(b)]

All necessary testing was conducted on BioCode Respiratory Pathogen Panel (RPP) to support a determination of substantial equivalence to the predicate device.

Nonclinical testing summary [807.92(b)(1)]

REPRODUCIBILITY STUDY

A study was performed to assess the Reproducibility of the BioCode® RPP using samples extracted with the KingFisher Flex. This study was designed to assess intra-assay (within run), Inter-assay (run-to-run), day-to-day and instrument-to-instrument (operator-to-operator) reproducibility. One lot of BioCode® RPP was assayed by 3 operators on 5 non-consecutive days. Each operator performed extraction using 2 KFF instruments daily for a combined total of 30 extractions. The extracted samples were tested on 3 MDx-3000 instruments with each operator performing 2 runs on each instrument across the 5 days.

Table 1. KingFisher Flex – Reproducibility Panel.

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

Table 2. Reproducibility Results

Analyte	Concentration Tested	Expected Result	Agreement with Expected Results
			KingFisher Flex Extraction (95% CI)
Viruses			
Adenovirus	**3× LoD**	**Detected**	**90/90 100%** (95.9%-100%)
	1.5× LoD	**Detected**	**90/90 100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**630/630 100%** (99.4%-100%)
Coronavirus NL63	**3× LoD**	**Detected**	**90/90 100%** (95.9%-100%)
	1.5× LoD	**Detected**	**90/90 100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**630/630 100%** (99.4%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Results
			KingFisher Flex Extraction (95% CI)
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	**3× LoD**	**Detected**	**89/90*** **98.9%** (94.0%-99.8%)
	1.5× LoD	**Detected**	**88/90** **97.8%** (92.3%-99.4%)
	None (no analyte)	**Not Detected**	**630/630** **100%** (99.4%-100%)
Human Rhinovirus/ Enterovirus	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**86/90** **95.6%** (89.1%-98.3%)
	None (no analyte)	**Not Detected**	**630/630** **100%** (99.4%-100%)
Influenza A/H3	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× 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	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**630/630** **100%** (99.4%-100%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Results
			KingFisher Flex Extraction (95% CI)
Parainfluenza Virus 1	**None** (no analyte)	**Not Detected**	**810/810** **100%** (99.6%-100%)
Parainfluenza Virus 2	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**88/90** **97.8%** (92.3%-99.4%)
	None (no analyte)	**Not Detected**	**630/630** **100%** (99.4%-100%)
Parainfluenza Virus 3	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× 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	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**629/630** **99.8%** (99.1%-100%)
Bacteria			
Mycoplasma pneumoniae	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**630/630** **100%** (99.4%-100%)
	3× LoD	**Detected**	**89/90*** **98.9%** (94.0%-99.8%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Results
			KingFisher Flex Extraction (95% CI)
Bordetella pertussis	**1.5× LoD**	**Detected**	**88/90** **97.8%** (92.3%-99.4%)
	None (no analyte)	**Not Detected**	**629/630** **99.8%** (99.1%-100%)
Chlamydia pneumoniae	**3× LoD**	**Detected**	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**89/90** **98.9%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**630/630** **100%** (99.4%-100%)

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

LIMIT OF DETECTION (LoD)

A study was conducted to evaluate the performance of the BioCode® RPP assay on the BioCode® MDx-3000 system using the KingFisher Flex extraction system. Quantified bacterial and viral stocks were spiked into simulated nasopharyngeal swab (NPS) specimens in universal transport medium (UTM). Initial limit of detection (LoD) estimates were determined by testing four replicates per concentration. LoD confirmation was performed by extracting and testing 20 replicates per sample type at or near the presumptive LoD. The LoD for each organism was defined as the lowest concentration detected in ≥95% of replicates (≥19/20).

Table 3. Limit of Detection for KingFisher Flex Extraction System.

Target	Species/Strain/Isolate	Source	KFF Concentration	Detected (n of 20)
Influenza A H1	A/New Caledonia/20/99	Zeptometrix 0810036CF	5.0 $TCID_{50}$/mL	20/20
	A/NWS/33	ATCC VR-219	27.0 $TCID_{50}$/mL	20/20
Influenza A H1 2009pdm	A(H1N1)/California/07/09	Zeptometrix 0810165CF	0.4 $TCID_{50}$/mL	20/20
Influenza A H3	A/Wisconsin/67/05a	Zeptometrix 0810252CF	1.3 $TCID_{50}$/mL	20/20
	A/Alice	ATCC VR 776	27.0 $CEID_{50}$/mL	20/20
Influenza B	Flu B/Florida/4/2006 (Yamagata)	Zeptometrix 0810255CF	0.06 $TCID_{50}$/mL	20/20
	B/Hong Kong/5/1972 (Victoria)	ATCC VR 823	5.4 $TCID_{50}$/mL	19/20
Respiratory Syncytial	Type A	Zeptometrix 0810040ACF	0.33 $TCID_{50}$/mL	20/20

Target	Species/Strain/Isolate	Source	KFF Concentration	Detected (n of 20)
Virus				
Human Metapneumovirus	16; Type A1 IA10-2003	Zeptometrix 0810161CF	1.67 $TCID_{50}$/mL	20/20
Parainfluenza Virus 1	C-35/Washington DC/1957	ATCC VR-94	9.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 2	Greer/Ohio/1955	ATCC VR-92	5.4 $TCID_{50}$/mL	19/20
Parainfluenza Virus 3	N/A	Zeptometrix 0810016CF	15.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 4	Type 4a	Zeptometrix 0810060CF	81.0 $TCID_{50}$/mL	20/20
Adenovirus	Species B Serotype 7A	Zeptometrix 0810021CF	3.6 $TCID_{50}$/mL	20/20
Adenovirus	Species C Serotype 2	ATCC VR-846	18.0 $TCID_{50}$/mL	20/20
Adenovirus	Species E Serotype 4	Zeptometrix 0810070CF	0.01 $TCID_{50}$/mL	19/20
Coronavirus 229E	N/A	Zeptometrix 0810229CF	1.8 $TCID_{50}$/mL	20/20
Coronavirus HKU1	N/A	Clinical Sample[a]	$1.5x10^5$ copies/mL	19/20
Coronavirus NL63	N/A	Zeptometrix 0810228CF	0.04 $TCID_{50}$/mL	20/20
Coronavirus OC43	N/A	Zeptometrix 0810024CF	0.09 $TCID_{50}$/mL	20/20
Rhinovirus	Rhinovirus Type A1	Zeptometrix 0810012CF	0.4 $TCID_{50}$/mL	20/20
Enterovirus	Enterovirus D68	Zeptometrix 0810300CF	9.0 $TCID_{50}$/mL	19/20
Bordetella pertussis	A639	Zeptometrix 0801459	270.0 CFU/mL	20/20
Chlamydia pneumoniae	AR-39	ATCC 53592	33.3 IFU/mL	20/20
Mycoplasma pneumoniae	M129	Zeptometrix 0801579	45.0 CCU/mL	20/20

a. Coronavirus HKU1 clinical sample quantified (copies/mL) with Applied BioCode validated SYBR assay using an IV RNA standard

Clinical testing summary [807.92(b)(2)]

METHOD COMPARISON STUDY

A clinical investigational study was performed in which a total of 735 (629 positive and 106 negative) archived nasopharyngeal swab (NPS) clinical samples collected from patients across five geographically diverse investigational sites as part of the original clinical study supporting the BioCode® RPP 510(k) clearance (K192485), as well as retrospective NPS clinical samples collected from clinical sample procurement vendors. Contrived clinical samples were tested for low-prevalence/rare analytes, twenty-five (25) at 2x LoD and twenty-five (25) at 4x LoD.

The demographics of the clinical patients and the results of the clinical investigational study are as follows:

Table 4. Demographic data for archived samples

Archived Samples	
Total Specimen Count	735
Gender	
Male	374/735 (50.4%)
Female	361/735 (49.1%)
Age Category	
≤ 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%)

A. Archived Clinical Sample Study

The positive percent agreement (PPA; [true positive/true positive + false negative] X 100), as well as the 95% exact binomial two-sided confidence interval, was calculated for the analytes that were part of the archived clinical sample study. All the targets tested showed a positive percent agreement (PPA) of at least 90%. The PPAs ranged from 90.7% to 100%.
The negative percent agreement (NPA; [true negative/true negative + false positive] X 100), as well as the 95% exact binomial two-sided confidence interval, was calculated for all analytes including those not enrolled in the archived clinical sample study due to low prevalence. All the targets tested showed a negative percent agreement (NPA) of at least 99%. The NPAs ranged from 99.1% to 100%.

Table 5. Results from archived samples tested with BioCode® RPP using KFF extracts compared to MP96 extracts

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	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%

Target	Positive Agreement		Negative Agreement	
	PPA (%)	95% CI	NPA (%)	95% CI
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%

Note: There were three invalid samples due to internal control below the cut-off. Two were for KFF extraction and one was for MP96. The PCR was repeated for the KFF invalid samples using the reserved extract and resolved. The MP96 invalid sample could not be repeated from the saved extract or re-extracted due to insufficient volume and was excluded from the analysis. The sample was positive by both extraction systems for RSV.

B. Contrived Sample Study

The PPA and NPA including 95% exact binomial two-sided confidence intervals were calculated for targets in the contrived sample study. All targets had 100% PPA (for both 2x and 4x LoD) and 100% NPA, except *Bordetella pertussis* which had 95.7% PPA at 2x LoD, 100% at 4x LoD and 99.2% NPA.

Table 6. Results from contrived samples tested by BioCode® RPP using the KFF extraction system compared to MP96

Target	Source	Strain/ Isolate	Fold LoD	Concentration	PA (%)	95% CI	NA (%)	95% CI
Bordetella pertussis	Zeptometrix 0801459	A639	2	$5.40x10^2$ CFU/mL	22/23* (95.7%)	79.0%-99.2%	249/251^ (99.2%)	97.1%-99.8%
			4	$1.08x10^3$ CFU/mL	25/25 (100%)	86.7%-100%		
	Combined				47/49 (95.9%)	86.3%-98.9%		
Chlamydia pneumoniae	ATCC 53592	TW-183 (AR39)	2	$6.67x10^1$ IFU/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
			4	$1.33x10^2$ IFU/mL	25/25 (100%)			
	Combined				50/50 (100%)	92.9%-100%		
Influenza A H1	Zeptometrix 0810036CF	A/New Caledonia/20/99	2	$1.00x10^1$ $TCID_{50}$/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
			4	$2.00x10^1$ $TCID_{50}$/mL	25/25 (100%)			
	Combined				50/50 (100%)	92.9%-100%		
Mycoplasma pneumoniae	Zeptometrix 0801579	M129	2	$9.00x10^1$ CCU/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
			4	$1.80x10^2$ CCU/mL	25/25 (100%)			
	Combined				50/50 (100%)	92.9%-100%		
Parainfluenza Virus 1	Zeptometrix 0810014CF	C-35/ Washington DC/1957	2	$1.80x10^1$ $TCID_{50}$/mL	25/25 (100%)	86.7%-100%	250/250 (100%)	98.5%-100%
			4	$3.60x10^1$ $TCID_{50}$/mL	25/25 (100%)			
	Combined				50/50 (100%)	92.9%-100%		
		Type 4a	2	$1.62x10^2$ $TCID_{50}$/mL	25/25 (100%)	86.7%-100%		

Parainfluenza Virus 4	Zeptometrix 0810060CF		4	$3.24x10^2$ $TCID_{50}$/mL	25/25 (100%)		250/250 (100%)	98.5%-100%
	Combined				50/50 (100%)	92.9%-100%		

*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.
^ BP was detected in one of the 4x LoD PIV4 MP96 extracted contrived samples

C. Additional Clinical Agreement Study

Because the Bordetella pertussis LOD using the KingFisher™Flex was >3x higher than the MagNA Pure 96 LOD, a supplemental study to the contrived study was performed. 10 retrospective clinical samples positive for B. pertussis were extracted using the KingFisher™Flex and MagNA Pure 96 and tested using the BioCode®RPP. The PPA was 100%.

Table 7. KingFisher™Flex *B. pertussis* clinical sample performance compared to MagNA Pure 96

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)

COMPETITIVE INHIBITION

A study was performed to evaluate the potential for inhibition in samples with mixed infections. Targets were spiked into simulated NPS in UTM matrix with one target at high concentration ($\geq 10^5$ units/mL) and two targets at low concentration (3x LoD). The study was performed for Adenovirus C serotype 2 and *B. pertussis* as the low target because these targets had KingFisher Flex LODs that were >3x higher than the current extractions systems. Testing was performed for the same common co- infections previously determined for the predicate device. Each sample was extracted in triplicate on the KingFisher Flex and each extraction tested in singlet with the BioCode® RPP. All replicates were detected and no competitive inhibition was observed.

Table 8. Competitive inhibition results for KingFisher™Flex

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	

Panel Designation	Viral/Bacterial Strain	Source	Level	Titer Tested	Detected (n of 3)	Pass/Fail
	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	

VIII. Conclusions [807.92(b)(3):

The intended use and fundamental scientific technology of the BioCode RPP using the KingFisher Flex is substantially equivalent to the predicate device. Non-clinical studies and clinical studies have established that the BioCode RPP with the KingFisher Flex is substantially equivalent to the predicate device.