Applied BioCode, Inc.
Robert Tullio
Regulatory Consultant
10020 Pioneer Blvd Suite 102
Santa Fe Springs, California 90670

December 23, 2019

Re: K192485
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, OZE, OEP, OEM, OOU, OTG, OZX, OZY, OZZ, NSU
Dated: December 5, 2019
Received: December 6, 2019

Dear Robert Tullio:

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 (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 located 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.

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 803) for devices or postmarketing safety reporting (21 CFR 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 systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 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,

Tamara Feldblyum, Ph.D.
Chief
Viral Respiratory and STI Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K192485

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 Virus 1
- Parainfluenza Virus 2
- Parainfluenza Virus 3
- Parainfluenza Virus 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 Rhinovirus/Enterovirus 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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1.0 510(k) SUMMARY

Introduction: According to the requirements of 21 CFR 807.92, the following provides information to understand the basis for a determination of substantial equivalence.

Submitted by:
Applied BioCode®, Inc.
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Santa Fe Springs, CA 90670

Contact:
Robert Di Tullio
Regulatory Consultant
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Telephone: 310 801 1235
Fax: 323 372 3816

Date Submitted:
September 9, 2019

Trade Name:
BioCode® Respiratory Pathogen Panel (RPP)

Classification Name and Regulation Number:
Respiratory Viral Panel Multiplex Nucleic Acid Assay (21 CFR 866.3980)

Predicate Device:
K170604 – BioFire FilmArray Respiratory Pathogen Panel 2 (RP2)

Intended Use:

BioCode® Respiratory Pathogen Panel (RPP)

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.

Device Description:

The BioCode® Respiratory Pathogen Panel 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 transport media. Specimens are processed and nucleic acids extracted with the NucliSens easyMAG or Roche MagNA Pure 96 automated systems. Once the PCR plate is set up and sealed, all other operations are automated on MDx-3000. The BioCode® RPP simultaneously tests for 17 pathogens and/or subtypes (see table below) from nasopharyngeal swab specimens collected in UTM or VTM. Results from the BioCode RPP test are available within about 5 hours, including off-board nucleic acids extraction.

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*
	Internal Control (MS2)

Device Comparison:

Comparison of the BioCode RPP with the Predicate Device

Characteristic	Proposed Device	Predicate
Name	BioCode® Respiratory Pathogen Panel (RPP)	BioFire FilmArray Respiratory Pathogen Panel 2 (RP2)
Common Name	Respiratory Pathogen Panel Multiplex Nucleic acid assay	Respiratory Viral Panel Multiplex Nucleic acid assay
510(k) No.	K192485	K170604
Regulation	21CFR 866.3980	21CFR 866.3980
Product Code	OCC, OZE, OEM, OOU, OEP, OTG, OZX, OZY, OZZ, NSU	OCC, OEM, OOU, OEP, OTG, OZX, OZY, OZZ, OOI
Device Class	II	II

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 • 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)	The FilmArray Respiratory Panel 2 (RP2) is a multiplexed nucleic acid test intended for use with FilmArray® 2.0 or FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP2: • Adenovirus • Coronavirus 229E • Coronavirus HKU1 • Coronavirus NL63 • Coronavirus OC43 • Human Metapneumovirus • Human Rhinovirus/Enterovirus • Influenza A, including subtypes H1, H1-2009, and H3 • Influenza B • Parainfluenza Virus 1 • Parainfluenza Virus 2 • Parainfluenza Virus 3 • Parainfluenza Virus 4 • Respiratory Syncytial Virus • *Bordetella parapertussis* (IS*1001*) • *Bordetella pertussis* (*ptxP*) • *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 FilmArray RP2 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and

	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.	radiography) may be necessary when evaluating a patient with possible respiratory tract infection. Due to the genetic similarity between Human Rhinovirus and Enterovirus, the FilmArray RP2 cannot reliably differentiate them. A positive FilmArray RP2 Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is required. Performance characteristics for Influenza A were established when Influenza A H1-2009, A H1, 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.
Sample Type	Nasopharyngeal swab in Viral or Universal Transport Media	Same
Controls	Externally Sourced	Same
Differences		
Methodology	Multiplex RT-PCR in a single reaction and probe hybridization followed by fluorescence detection and decoding of barcoded magnetic beads (BMB) that capture biotinylated products with streptavidin conjugate	Nested multiplex PCR executed in two stages. First, a single, large volume, highly multiplexed reverse transcription PCR (RT-PCR) reaction. Second, nested PCR, is performed in singleplex fashion in each well of the array. Followed by fluorescent detection of images of the PCR reactions.

Summary of Performance Characteristics of the BioCode RPP®

Clinical Performance

Study Overview

The clinical performance of the BioCode RPP was established in a multi-center study conducted during periods of the 2017-2019 respiratory illness seasons. Residual (leftover) and de-identified nasopharyngeal swab (NPS) specimens in VTM or UTM that were prospectively collected from patients suspected of respiratory tract infections at five geographically diverse clinical sites in the U.S. were enrolled and tested with the BioCode RPP at five testing sites during the prospective clinical study. The enrolled prospective specimens were tested freshly with an FDA-cleared molecular multiplexed respiratory pathogen panel as part of the Stand of Care (SOC), and were either tested freshly with the BioCode RPP (i.e., specimens that were stored in a 2-8°C refrigerator for no more than 7 days), or stored frozen and then thawed and tested with the BioCode RPP at a testing site at a later date (i.e., specimens that were initially stored in a 2-8°C refrigerator but were not able to be tested by the BioCode RPP within 7 days from specimen collection).

A waiver of the informed consent requirement was obtained from the Institutional Review Boards (IRBs) at each specimen enrollment site for the use of residual NPS in VTM or UTM specimens.

The following information was recorded on the Case Report Form (CRF) for each subject from whom a specimen was enrolled:

- Age and sex
- Date and time of specimen collection
- Standard of care (SOC) comparator test result
- Specimen storage status, i.e., fresh or frozen

A total of 2654 residual NPS specimens in VTM or UTM that were prospectively collected at the five clinical sites from August 2017 to May 2019 were enrolled initially for the clinical study. Five specimens were withdrawn from the clinical study due to incomplete data collection and testing, resulted in a total of 2649 prospective specimens (1401 fresh and 1248 frozen specimens) that were included in the prospective clinical study.

The prospective specimens enrolled for evaluation were tested at the five testing sites by trained laboratory personnel. DNA/RNA was extracted using either the BioMerieux NucliSENS easyMAG system or Roche MagNA Pure 96 system. After extraction, the samples were tested using the BioCode RPP on the BioCode MDx-3000 System according to the instructions for use.

Demographics – Prospective Samples

Characteristic	Prospective Study Specimens
Total Specimens	2649
Gender (n/N (%))	
Male	1346/2649 (50.8%)

Characteristic	Prospective Study Specimens
Female	1303/2649 (49.2%)
Age Category (n/N (%))	
0-5 yrs	1004/2649 (37.9%)
6-21 yrs	609/2649 (23.0%)
22-59 yrs	531/2649 (20.0%)
60+ yrs	505/2649 (19.1%)
Status (n/N (%))[a]	
Inpatient	1555/2646 (58.8%)
Outpatient	1091/2646 (41.2%)

a – Inpatient/outpatient status was unavailable for 3 specimens.

Prospective Sample Type and Test Method Breakdown

		Storage Method		Extraction Method	
Site	**Samples Tested**	**Fresh**	**Frozen**	**easyMAG**	**MagNA Pure 96**
Site 01	530	250	280	530	0
Site 02	419	182	237	0	419
Site 03	600	366	234	600	0
Site 04	550	300	250	550	0
Site 05	550	303	247	0	550
Total:	**2649**	**1401**	**1248**	**1680**	**969**

Performance of the BioCode RPP was evaluated by comparing the BioCode RPP test results with those from an FDA-cleared molecular multiplexed respiratory pathogen panel. Positive agreement was calculated as TP/(TP + FN). TP = true positive or positive by both the comparator test and BioCode RPP; FN = false negative or negative by BioCode RPP only. Negative agreement was calculated as TN/(TN + FP). TN = true negative or negative by both the comparator test and BioCode RPP; FP = false positive or positive by BioCode RPP only. The two-sided 95% confidence interval was calculated with Score method (per CLSI EP12-A2).

Samples for which false positive and/or false negative results (i.e., discrepant results) were obtained when comparing the BioCode RPP results to the comparator test results were further investigated. The discrepancy investigation was mainly conducted by performing independent molecular tests, including analytically validated PCR followed by bi-directional sequencing assays and alternate NAATs.

Of the 2649 specimens included in the prospective clinical study, two specimens, one fresh and one frozen specimen, obtained a final "invalid" result from the BioCode RPP, and were excluded from the performance analyses for all analytes. In addition, three specimens, one fresh and two frozen specimens, obtained a final influenza A "indeterminate" result by the BioCode RPP, and two specimens, one fresh and one frozen, obtained an influenza A "equivocal" result from the comparator test. They were included in the performance analyses for all analytes but excluded from the performance

calculations for Flu A and Flu A subtypes. Furthermore, two frozen specimens obtained a valid influenza A result from the comparator test without the accompanying Flu A subtyping results. They were included in the performance analyses for all analytes but excluded from the performance calculations for Flu A subtypes.

The Prospective study results stratified by storage condition are presented in the table below.

Table. Summary of Clinical Study results: Prospective specimens stratified by storage condition.

			Positive Agreement		Negative Agreement	
Target	**Storage**	**(n)**	**PA (%)**	**95% CI**	**NA (%)**	**95% CI**
Adenovirus[a]	Fresh	1400	31/40 (77.5%)	(62.5%, 87.7%)	1340/1360 (98.5%)	(97.7%, 99.0%)
	Frozen	1247	37/38 (97.4%)	(86.5%, 99.5%)	1188/1209 (98.3%)	(97.4%, 98.9%)
	Total	**2647**	**68/78 (87.2%)**	**(78.0%, 92.9%)**	**2528/2569 (98.4%)**	**(97.8%, 98.8%)**
Bordetella pertussis[b]	Fresh	1400	1/1 (100%)	(20.7%, 100%)	1387/1399 (99.1%)	(98.5%, 99.5%)
	Frozen	1247	1/1 (100%)	(20.7%, 100%)	1239/1246 (99.4%)	(98.8%, 99.7%)
	Total	**2647**	**2/2 (100%)**	**(34.2%, 100%)**	**2626/2645 (99.3%)**	**(98.9%, 99.5%)**
Chlamydia pneumoniae[c]	Fresh	1400	2/2 (100%)	(34.2%, 100%)	1397/1398 (99.9%)	(99.6%, 100%)
	Frozen	1247	2/2 (100%)	(34.2%, 100%)	1245/1245 (100%)	(99.7%, 100%)
	Total	**2647**	**4/4 (100%)**	**(51.0%, 100%)**	**2642/2643 (100%)**	**(99.8%, 100%)**
Coronavirus[d]	Fresh	1400	35/50 (70%)	(56.2%, 80.9%)	1338/1350 (99.1%)	(98.5%, 99.5%)
	Frozen	1247	76/83 (91.6%)	(83.6%, 95.9%)	1154/1164 (99.1%)	(98.4%, 99.5%)
	Total	**2647**	**111/133 (83.5%)**	**(76.2%, 88.8%)**	**2492/2514 (99.1%)**	**(98.7%, 99.4%)**
Human Metapneumovirus[e]	Fresh	1400	89/93 (95.7%)	(89.5%, 98.3%)	1299/1307 (99.4%)	(98.8%, 99.7%)
	Frozen	1247	46/49 (93.9%)	(83.5%, 97.9%)	1189/1198 (99.2%)	(98.6%, 99.6%)
	Total	**2647**	**135/142 (95.1%)**	**(90.2%, 97.6%)**	**2488/2505 (99.3%)**	**(98.9%, 99.6%)**
Human Rhinovirus/Enterovirus[f]	Fresh	1400	221/261 (84.7%)	(79.8%, 88.5%)	1119/1139 (98.2%)	(97.3%, 98.9%)
	Frozen	1247	162/213 (76.1%)	(69.9%, 81.3%)	1020/1034 (98.6%)	(97.7%, 99.2%)
	Total	**2647**	**383/474 (80.8%)**	**(77.0%, 84.1%)**	**2139/2173 (98.4%)**	**(97.8%, 98.9%)**
Influenza A[g]	Fresh	1398	115/120 (95.8%)	(90.6%, 98.2%)	1265/1278 (99.0%)	(98.3%, 99.4%)
	Frozen	1244	98/101 (97.0%)	(91.6%, 99.0%)	1131/1143 (99.0%)	(98.2%, 99.4%)
	Total	**2642**	**213/221 (96.4%)**	**(93.0%, 98.2%)**	**2396/2421 (99.0%)**	**(98.5%, 99.3%)**
Influenza A H1	Fresh	1398	N/A[†]	N/A[†]	1398/1398 (100%)	(99.7%, 100%)
	Frozen	1242	N/A[†]	N/A[†]	1242/1242 (100%)	(99.7%, 100%)
	Total	**2640**	**N/A[†]**	**N/A[†]**	**2640/2640 (100%)**	**(99.9%, 100%)**
Influenza A H1 2009pdm[h]	Fresh	1398	29/30 (96.7%)	(83.3%, 99.4%)	1365/1368 (99.8%)	(99.4%, 99.9%)
	Frozen	1242	23/23 (100%)	(85.7%, 100%)	1213/1219 (99.5%)	(98.9%, 99.8%)
	Total	**2640**	**52/53 (98.1%)**	**(90.1%, 99.7%)**	**2578/2587 (99.7%)**	**(99.3%, 99.8%)**

			Positive Agreement		Negative Agreement	
Target	**Storage**	**(n)**	**PA (%)**	**95% CI**	**NA (%)**	**95% CI**
Influenza A H3[i]	Fresh	1398	82/88 (93.2%)	(85.9%, 96.8%)	1306/1310 (99.7%)	(99.2%, 99.9%)
	Frozen	1242	65/69 (94.2%)	(86.0%, 97.7%)	1168/1173 (99.6%)	(99.0%, 99.8%)
	Total	**2640**	**147/157 (93.6%)**	**(88.7%, 96.5%)**	**2474/2483 (99.6%)**	**(99.3%, 99.8%)**
Influenza B[j]	Fresh	1400	7/7 (100%)	(64.6%, 100%)	1388/1393 (99.6%)	(99.2%, 99.8%)
	Frozen	1247	44/47 (93.6%)	(82.8%, 97.8%)	1191/1200 (99.2%)	(98.6%, 99.6%)
	Total	**2647**	**51/54 (94.4%)**	**(84.9%, 98.1%)**	**2579/2593 (99.5%)**	**(99.1%, 99.7%)**
Mycoplasma pneumoniae[k]	Fresh	1400	8/8 (100%)	(67.6%, 100%)	1381/1392 (99.2%)	(98.6%, 99.6%)
	Frozen	1247	10/10 (100%)	(72.2%, 100%)	1228/1237 (99.3%)	(98.6%, 99.6%)
	Total	**2647**	**18/18 (100%)**	**(82.4%, 100%)**	**2609/2629 (99.2%)**	**(98.8%, 99.5%)**
Parainfluenza Virus 1[l]	Fresh	1400	4/4 (100%)	(51.0%, 100%)	1396/1396 (100%)	(99.7%, 100%)
	Frozen	1247	11/13 (84.6%)	(57.8%, 95.7%)	1234/1234 (100%)	(99.7%, 100%)
	Total	**2647**	**15/17 (88.2%)**	**(65.7%, 96.7%)**	**2630/2630 (100%)**	**(99.9%, 100%)**
Parainfluenza Virus 2[m]	Fresh	1400	2/3 (66.7%)	(20.8%, 93.9%)	1396/1397 (99.9%)	(99.6%, 100%)
	Frozen	1247	8/9 (88.9%)	(56.5%, 98.0%)	1236/1238 (99.8%)	(99.4%, 100%)
	Total	**2647**	**10/12 (83.3%)**	**(55.2%, 95.3%)**	**2632/2635 (99.9%)**	**(99.7%, 100%)**
Parainfluenza Virus 3[n]	Fresh	1400	77/79 (97.5%)	(91.2%, 99.3%)	1312/1321 (99.3%)	(98.7%, 99.6%)
	Frozen	1247	41/43 (95.3%)	(84.5%, 98.7%)	1196/1204 (99.3%)	(98.7%, 99.7%)
	Total	**2647**	**118/122 (96.7%)**	**(91.9%, 98.7%)**	**2508/2525 (99.3%)**	**(98.9%, 99.6%)**
Parainfluenza Virus 4[o]	Fresh	1400	1/1 (100%)	(20.7%, 100%)	1399/1399 (100%)	(99.7%, 100%)
	Frozen	1247	15/17 (88.2%)	(65.7%, 96.7%)	1228/1230 (99.8%)	(99.4%, 100%)
	Total	**2647**	**16/18 (88.9%)**	**(67.2%, 96.9%)**	**2627/2629 (99.9%)**	**(99.7%, 100%)**
Respiratory Syncytial Virus[p]	Fresh	1400	91/93 (97.8%)	(92.5%, 99.4%)	1293/1307 (98.9%)	(98.2%, 99.4%)
	Frozen	1247	109/111 (98.2%)	(93.7%, 99.5%)	1129/1136 (99.4%)	(98.7%, 99.7%)
	Total	**2647**	**200/204 (98.0%)**	**(95.1%, 99.2%)**	**2422/2443 (99.1%)**	**(98.7%, 99.4%)**

[†] *No positive reference results recorded*

a – Adenovirus: The 10 FNs were not detected by PCR/bi-directional sequencing or alternative NAAT. Of 41 FPs, 37 were not detected and 4 were indeterminate by PCR/bi-directional sequencing.

b – *Bordetella pertussis:* Of 19 FPs, 4 were detected by PCR/bi-directional sequencing, 3 were indeterminate and 12 were not detected by PCR/bi-directional sequencing.

c – *Chlamydia pneumoniae:* The 1 FP was detected by PCR/bi-directional sequencing.

d – Coronavirus: Of 22 FNs, 5 were detected by PCR/bi-directional sequencing. 2 were not detected by PCR/bi-directional sequencing but detected by alternative NAAT. 12 were not detected by either alternative NAAT or PCR/bi-directional sequencing. 3 were not detected by PCR/bi-directional sequencing and were not tested by alternative NAAT. The 22 FPs were not detected by PCR/bi-directional sequencing.

e – Human Metapneumovirus: Of 7 FNs, 3 were detected and 4 were not detected by PCR/bi-directional sequencing. Of 17 FPs, 7 were detected while 10 were not detected by PCR/bi-directional sequencing.

f – Human Rhinovirus/Enterovirus: Of 91 FNs, 26 were detected, and 2 were indeterminate by PCR/bi-directional sequencing. 25 were not detected by PCR/bi-directional sequencing but were detected by alternative NAAT. 11 were not detected by either PCR/bi-directional

sequencing or by alternative NAAT. 27 were not detected by PCR/bi-directional sequencing and had insufficient volume for alternative NAAT. Of 34 FPs, 8 were detected and 26 were not detected by PCR/bi-directional sequencing.

g – Influenza A: Of the 8 FNs, 7 were not detected by PCR/bi-directional sequencing. 1 had insufficient volume for follow-up testing. Of 25 FPs, 5 were detected by PCR/bi-directional sequencing, 19 were not detected by PCR/bi-directional sequencing, and 1 had insufficient volume for follow-up testing.

h – Influenza A H1 2009pdm: The 1 FN had insufficient volume for follow-up testing. Of 9 FPs, 4 were detected by PCR/bi-directional sequencing, and 5 were not detected by PCR/bi-directional sequencing.

i – Influenza A H3: Of 10 FNs, 7 were not detected and 1 was detected by PCR/bi-directional sequencing. 2 had insufficient volume for follow-up testing. Of 9 FPs, 3 were detected, 1 was indeterminate, and 4 were not detected by PCR/bi-directional sequencing. 1 had insufficient volume for follow-up testing.

j – Influenza B: The 3 FNs were not detected by PCR/bi-directional sequencing. Of 14 FPs, 1 was detected and 12 were not detected by PCR/bi-directional sequencing, and 1 had insufficient volume for follow-up testing.

k – *Mycoplasma pneumoniae:* Of 20 FPs, 7 were detected, 10 were not detected, and 1 was invalid by PCR/bi-directional sequencing. 2 had insufficient volume for follow-up testing.

l – Parainfluenza Virus 1: The 2 FNs were not detected by PCR/bi-directional sequencing.

m – Parainfluenza Virus 2: Of 2 FNs, 1 was detected and 1 was not detected by PCR/bi-directional sequencing. The 3 FPs were detected by PCR/bi-directional sequencing.

n – Parainfluenza Virus 3: Of 4 FNs, 2 were detected, 1 was not detected, and 1 was indeterminate, by PCR/bi-directional sequencing. Of 17 FPs, 8 were detected, 8 were not detected, and 1 was indeterminate, by PCR/bi-directional sequencing.

o – Parainfluenza Virus 4: The 2 FNs were detected by PCR/bi-directional sequencing. The 2 FPs were detected by PCR/bi-directional sequencing.

p – Respiratory Syncytial Virus: The 4 FNs were not detected by PCR/bi-directional sequencing. Of 21 FPs, 18 were not detected, 2 were detected, and 1 was indeterminate, by PCR/bi-directional sequencing.

Prospective prevalence as detected by BioCode® RPP, stratified by age or study site, are presented in tables below.

Table. Prospective prevalence detected by BioCode® RPP stratified by Age

Analyte	Overall (N=2647)	≤5 yrs (N=1004)	6-21 yrs (N=609)	22-59 yrs (N=531)	60+ yrs (N=503)
Adenovirus	109 (4.1%)	80 (8.0%)	20 (3.3%)	5 (0.9%)	4 (0.8%)
Bordetella pertussis	21 (0.8%)	8 (0.8%)	12 (2.0%)	0 (0.0%)	1 (0.2%)
Chlamydia pneumoniae	5 (0.2%)	1 (0.1%)	3 (0.5%)	1 (0.2%)	0 (0.0%)
Coronavirus	133 (5.0%)	63 (6.3%)	27 (4.4%)	19 (3.6%)	24 (4.8%)
Human Metapneumovirus	152 (5.7%)	94 (9.4%)	26 (4.3%)	15 (2.8%)	17 (3.4%)
Human Rhinovirus/Enterovirus	417 (15.8%)	234 (23.3%)	101 (16.6%)	51 (9.6%)	31 (6.2%)
Influenza A	238 (9.0%)	71 (7.1%)	84 (13.8%)	47 (8.9%)	36 (7.2%)
Influenza A H1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Influenza A H1 2009pdm	62 (2.3%)	24 (2.4%)	15 (2.5%)	15 (2.8%)	8 (1.6%)
Influenza A H3	157 (5.9%)	45 (4.5%)	61 (10.0%)	26 (4.9%)	25 (5.0%)
Influenza B	65 (2.5%)	13 (1.3%)	26 (4.3%)	15 (2.8%)	11 (2.2%)
Mycoplasma pneumoniae	38 (1.4%)	9 (0.9%)	21 (3.4%)	6 (1.1%)	2 (0.4%)
Parainfluenza Virus 1	15 (0.6%)	4 (0.4%)	1 (0.2%)	4 (0.8%)	6 (1.2%)
Parainfluenza Virus 2	13 (0.5%)	6 (0.6%)	4 (0.7%)	3 (0.6%)	0 (0.0%)
Parainfluenza Virus 3	135 (5.1%)	74 (7.4%)	21 (3.4%)	21 (4.0%)	19 (3.8%)

Analyte	Overall (N=2647)	≤5 yrs (N=1004)	6-21 yrs (N=609)	22-59 yrs (N=531)	60+ yrs (N=503)
Parainfluenza Virus 4	18 (0.7%)	12 (1.2%)	1 (0.2%)	2 (0.4%)	3 (0.6%)
Respiratory Syncytial Virus	221 (8.3%)	156 (15.5%)	30 (4.9%)	19 (3.6%)	16 (3.2%)

Table. Prospective prevalence detected by BioCode® RPP stratified by site.

Analyte	Overall (N=2647)	Site 1 (N=529)	Site 2 (N=419)	Site 3 (N=599)	Site 4 (N=550)	Site 5 (N=550)
Adenovirus	109 (4.1%)	8 (1.5%)	27 (6.4%)	21 (3.5%)	17 (3.1%)	36 (6.5%)
Bordetella pertussis	21 (0.8%)	0 (0.0%)	19 (4.5%)	1 (0.2%)	0 (0.0%)	1 (0.2%)
Chlamydia pneumoniae	5 (0.2%)	0 (0.0%)	3 (0.7%)	0 (0.0%)	1 (0.2%)	1 (0.2%)
Coronavirus	133 (5.0%)	36 (6.8%)	21 (5.0%)	14 (2.3%)	26 (4.7%)	36 (6.5%)
Human Metapneumovirus	152 (5.7%)	18 (3.4%)	22 (5.3%)	26 (4.3%)	38 (6.9%)	48 (8.7%)
Human Rhinovirus/Enterovirus	417 (15.8%)	48 (9.1%)	83 (19.8%)	65 (10.9%)	125 (22.7%)	96 (17.5%)
Influenza A	238 (9.0%)	49 (9.3%)	50 (11.9%)	35 (5.8%)	41 (7.5%)	63 (11.5%)
Influenza A H1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Influenza A H1 2009pdm	62 (2.3%)	13 (2.5%)	7 (1.7%)	11 (1.8%)	22 (4.0%)	9 (1.6%)
Influenza A H3	157 (5.9%)	32 (6.0%)	40 (9.5%)	19 (3.2%)	17 (3.1%)	49 (8.9%)
Influenza B	65 (2.5%)	12 (2.3%)	32 (7.6%)	15 (2.5%)	2 (0.4%)	4 (0.7%)
Mycoplasma pneumoniae	38 (1.4%)	4 (0.8%)	15 (3.6%)	5 (0.8%)	3 (0.5%)	11 (2.0%)
Parainfluenza Virus 1	15 (0.6%)	4 (0.8%)	0 (0.0%)	11 (1.8%)	0 (0.0%)	0 (0.0%)
Parainfluenza Virus 2	13 (0.5%)	0 (0.0%)	0 (0.0%)	3 (0.5%)	8 (1.5%)	2 (0.4%)
Parainfluenza Virus 3	135 (5.1%)	12 (2.3%)	28 (6.7%)	41 (6.8%)	9 (1.6%)	45 (8.2%)
Parainfluenza Virus 4	18 (0.7%)	0 (0.0%)	1 (0.2%)	9 (1.5%)	5 (0.9%)	3 (0.5%)
Respiratory Syncytial Virus	221 (8.3%)	21 (4.0%)	35 (8.4%)	25 (4.2%)	49 (8.9%)	91 (16.5%)

The overall success rate for initial specimen testing in the prospective study was 98.8% (2618/2649) (95% CI: 98.3% - 99.2%); 31 tests were unsuccessful (26 tests with an invalid result and 5 tests due to low BMB count/instrument error). Upon a single retest per the instructions for use, 29 of the 31 initially unsuccessful specimens generated a valid result. The final validity rate was 99.9% (2647/2649) (95% CI: 99.7%-100%).

There were 193 samples with mixed infections by the BioCode® RPP in the prospective clinical study (193/2649 or 7.3%). The distribution, prevalence and most common co-infections combinations detected by BioCode RPP in the prospective clinical study are summarized in the tables below.

Table. Distribution of co-infection combinations detected by BioCode® RPP from prospective clinical study

Analytes Detected Simultaneously	Number of Specimen
2	168 (87.0%)
3	24 (12.4%)
5	1 (0.5%)
Total Co-Infections	**193**

Table. Prevalence of targets in co-infection combinations detected by BioCode® RPP from prospective clinical study

Analyte	Prevalence in Co-Infections (N=193)
Adenovirus	51 (26.4%)
Bordetella pertussis	14 (7.3%)
Chlamydia pneumoniae	1 (0.5%)
Coronavirus	47 (24.4%)
Human Metapneumovirus	35 (18.1%)
Human Rhinovirus/Enterovirus	101 (52.3%)
Influenza A	37 (19.2%)
Influenza B	8 (4.1%)
Mycoplasma pneumoniae	7 (3.6%)
Parainfluenza Virus 1	2 (1.0%)
Parainfluenza Virus 2	4 (2.1%)
Parainfluenza Virus 3	39 (20.2%)
Parainfluenza Virus 4	8 (4.1%)
Respiratory Syncytial Virus	59 (30.1%)

Table. Most prevalent multiple detection combinations (5 or more instances) detected by BioCode® RPP from prospective clinical study.

Co-Infection Combination	Number of Specimen
Human Rhinovirus/Enterovirus + Respiratory Syncytial Virus	17
Adenovirus + Human Rhinovirus/Enterovirus	13
Human Metapneumovirus + Human Rhinovirus/Enterovirus	13
Human Rhinovirus/Enterovirus + Influenza A	11
Adenovirus + Respiratory Syncytial Virus	10
Human Rhinovirus/Enterovirus + Parainfluenza Virus 3	10
Coronavirus + Human Rhinovirus/Enterovirus	8
Coronavirus + Respiratory Syncytial Virus	7
Bordetella pertussis + Human Rhinovirus/Enterovirus	6

Co-Infection Combination	Number of Specimen
Adenovirus + Parainfluenza Virus 3	5
Coronavirus + Human Metapneumovirus	5
Coronavirus + Influenza A	5

Testing of Preselected Archived Specimens

Some of the pathogens on the BioCode RPP were of low prevalence and were not encountered in sufficiently large numbers during the prospective study to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective specimens was performed. These specimens were archived NPS in VTM or UTM specimens that were selected because they had previously tested positive for one of the following pathogens at the source laboratory: coronavirus 229E, coronavirus HKU1, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 4, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*, or had been negative in previous laboratory testing.

A total of 165 clinical specimens were enrolled for testing in this retrospective study. The specimens were randomized such that the users performing the BioCode RPP assay were blinded to the expected test result and shipped to one of two of the testing sites participated in the prospective clinical study for testing.

A summary of the demographic information of the tested samples is provided in the following table:

Demographics of Archived Specimens

Characteristic	Archived Study Specimens
Total Specimens	165
Gender (n/N (%))	
Male	96/165 (58.2%)
Female	69/165 (41.8%)
Age Category (n/N (%))	
0-5 yrs	77/165 (46.7%)
6-21 yrs	58/165 (35.2%)
22-59 yrs	15/165 (9.1%)
60+ yrs	15/165 (9.1%)

The performance of the BioCode RPP was evaluated by comparing the BioCode RPP test results with those from an FDA-cleared molecular multiplexed respiratory pathogen panel, the same panel test as the one used as the comparator in the prospective clinical study. The BioCode RPP retrospective performance data expressed as positive percent and negative percent agreements against the comparator method are presented by pathogen in the table below.

Table. Results from Archived Specimens tested by the BioCode® RPP with easyMAG extraction system.

Target	(n)	Positive Agreement		Negative Agreement	
		PA (%)	95% CI	NA (%)	95% CI
Adenovirus[a]	165	7/7 (100%)	(64.6%, 100%)	155/158 (98.1%)	(94.6%, 99.4%)
Bordetella pertussis[b]	165	10/10 (100%)	(72.2%, 100%)	144/155 (92.9%)	(87.7%, 96.0%)
Chlamydia pneumoniae	165	10/10 (100%)	(72.2%, 100%)	155/155 (100%)	(97.6%, 100%)
Coronavirus[c]	165	52/59 (88.1%)	(77.5%, 94.1%)	99/106 (93.4%)	(87.0%, 96.8%)
Human Metapneumovirus	165	4/4 (100%)	(51.0%, 100%)	161/161 (100%)	(97.7%, 100%)
Human Rhinovirus/Enterovirus[d]	165	16/23 (69.6%)	(49.1%, 84.4%)	141/142 (99.3%)	(96.1%, 99.9%)
Influenza A	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza A H1	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza A H1 2009pdm	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza A H3	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza B[e]	165	2/3 (66.7%)	(20.8%, 93.9%)	162/162 (100%)	(97.7%, 100%)
Mycoplasma pneumoniae[f]	165	7/7 (100%)	(64.6%, 100%)	153/158 (96.8%)	(92.8%, 98.6%)
Parainfluenza Virus 1[g]	165	12/13 (92.3%)	(66.7%, 98.6%)	152/152 (100%)	(97.5%, 100%)
Parainfluenza Virus 2[h]	165	19/20 (95%)	(76.4%, 99.1%)	144/145 (99.3%)	(96.2%, 99.9%)
Parainfluenza Virus 3	165	1/1 (100%)	(20.7%, 100%)	164/164 (100%)	(97.7%, 100%)
Parainfluenza Virus 4[i]	165	14/15 (93.3%)	(70.2%, 98.8%)	150/150 (100%)	(97.5%, 100%)
Respiratory Syncytial Virus[j]	165	11/12 (91.7%)	(64.6%, 98.5%)	152/153 (99.3%)	(96.4%, 99.9%)

[†] *No positive reference results recorded*

a - Adenovirus: Of 3 FPs, 1 was detected by PCR/bi-directional sequencing and 2 were not detected by PCR/bi-directional sequencing.

b - *Bordetella pertussis*: Of 11 FPs, 7 were detected by PCR/bi-directional sequencing and 4 were not detected by PCR/bi-directional sequencing.

c - Coronavirus: Of 7 FNs, 4 were detected by PCR/bi-directional sequencing while 3 were not detected by PCR/bi-directional sequencing. The 7 FPs were not detected by PCR/bi-directional sequencing. All had low MFIs (<620) near the MFI cut-off on initial BioCode RPP results.

d - Human Rhinovirus/Enterovirus: The 7 FNs were not detected by PCR/bi-directional sequencing. The 1 FP was not detected by PCR/bi-directional sequencing.

e - Influenza B: 1 FN was not detected by PCR/bi-directional sequencing.

f - *Mycoplasma pneumoniae*: Of 5 FPs, 3 were detected by PCR/bi-directional sequencing and 2 were not detected by PCR/bi-directional sequencing.

g - Parainfluenza Virus 1: 1 FN was not detected by PCR/bi-directional sequencing.

h - Parainfluenza Virus 2: 1 FN was not detected by PCR/bi-directional sequencing. 1 FP was not detected by PCR/bi-directional sequencing.

i - Parainfluenza Virus 4: 1 FN was not detected by PCR/bi-directional sequencing.

j - Respiratory Syncytial Virus: 1 FN was not detected by PCR/bi-directional sequencing. 1 FP was not detected by PCR/bi-directional sequencing.

Testing of Contrived Specimens

Some analytes are so rare that both prospective and archived specimen collection efforts were insufficient to demonstrate the clinical performance. To supplement the prospective and archived data, an evaluation of contrived specimens was performed for two pathogens: *Chlamydia pneumoniae* and Influenza A H1. These contrived clinical specimens were prepared using 50 unique natural NPS in VTM or UTM specimens that were previously tested negative for all BioCode RPP analytes. Contrived specimens were spiked at concentrations of 2X LOD or greater using different strains for each pathogen. The 50 positive samples of each pathogen were prepared, interspersed with negative samples and randomized before testing at one of the five testing sites participated in the prospective clinical study. A total of 110 samples, including 100 positives, were tested. The results of the BioCode RPP testing are presented in the following table:

Table. Results from contrived samples tested by the BioCode® RPP using the easyMAG extraction system.

Organism	Source	Strain/Isolate	Fold LoD	Concentration	PA (%)	95% CI	NA (%)	95% CI
Chlamydia pneumoniae	ATCC 53592	AR-39	2	33.4 CFU/mL	9/9 (100%)	70.1%, 100%	60/60 (100%)	94.0%, 100%
			10	167 CFU/mL	5/5 (100%)	56.6%, 100%		
			100	1670 CFU/mL	4/4 (100%)	51.0%, 100%		
	ATCC VR-1360	CM-1	2	33.4 CFU/mL	8/8 (100%)	67.6%, 100%		
			10	167 CFU/mL	5/5 (100%)	56.6%, 100%		
			100	1670 CFU/mL	3/3 (100%)	43.9%, 100%		
	ATCC VR-1310	CWL-029	2	33.4 CFU/mL	8/8 (100%)	67.6%, 100%		
			10	167 CFU/mL	5/5 (100%)	56.6%, 100%		
			100	1670 CFU/mL	3/3 (100%)	43.9%, 100%		
				Combined	50/50 (100%)	92.9%, 100%		
Influenza A H1N1	Zeptometrix 0810036CF	A/New Caledonia/20/99	2	30 $TCID_{50}$/ mL	5/5 (100%)	56.6%, 100%	60/60 (100%)	94.0%, 100%
			10	150 $TCID_{50}$/ mL	3/3 (100%)	43.9%, 100%		
			100	1500 $TCID_{50}$/ mL	3/3 (100%)	43.9%, 100%		
	Zeptometrix 0810247CF	A/Taiwan/42/06	2	30 $TCID_{50}$/ mL	5/5 (100%)	56.6%, 100%		
			10	150 $TCID_{50}$/ mL	3/3 (100%)	43.9%, 100%		
			100	1500 $TCID_{50}$/ mL	2/2 (100%)	34.2%, 100%		
	Zeptometrix 0810246CF	Singapore/63/04	2	30 $TCID_{50}$/ mL	5/5 (100%)	56.6%, 100%		
			10	150 $TCID_{50}$/ mL	2/2 (100%)	34.2%, 100%		
			100	1500 $TCID_{50}$/ mL	2/2 (100%)	34.2%, 100%		
	Virapur	A/Denver/1/1957	2	30 $TCID_{50}$/ mL	5/5 (100%)	56.6%, 100%		
			10	150 $TCID_{50}$/ mL	2/2 (100%)	34.2%, 100%		
			100	1500 $TCID_{50}$/ mL	2/2 (100%)	34.2%, 100%		
	ATCC VR-219	A/NWS/33	2	54 $TCID_{50}$/ mL	5/5 (100%)	56.6%, 100%		
			10	270 $TCID_{50}$/ mL	3/3 (100%)	43.9%, 100%		
			100	2700 $TCID_{50}$/ mL	3/3 (100%)	43.9%, 100%		
				Combined	50/50 (100%)	92.9%, 100%		

Performance of External Controls during Clinical Trials

During clinical evaluation of the BioCode® RPP, at least one negative control (NC) was included in each run. The negative control was UTM that was taken through all steps (extraction, amplification, and detection). It was recommended that external controls consisting of 5 pools of inactivated organism from ZeptoMetrix (catalog no. NATRPP-ABC) be assayed on a rotating basis.

Table. Recommended positive control pools formulation for inactivated organism from ZeptoMetrix.

Pool	Panel Member	Strain	Dilution Factor
PC-A	Influenza B	B/Florida/02/06	1/4
	Respiratory Syncytial Virus A	N/A	1/4
	Parainfluenza virus Type 1	N/A	1/4
	Rhinovirus 1A	N/A	1/4
PC-B	HKU1 Construct	N/A	1/4
	Parainfluenza virus Type 2	N/A	1/4
	Metapneumovirus 8	Peru6-2003	1/4
	Bordetella pertussis	A639	1/4
PC-C	Influenza A H1N1	A/New Caledonia/20/99	1/4
	Coronavirus NL63	N/A	1/4
	Adenovirus Type 3	N/A	1/4
PC-D	Influenza A 2009 H1N1pdm	A/NY/02/09	1/4
	Parainfluenza virus Type 4	N/A	1/4
	Coronavirus OC43	N/A	1/4
	Chlamydia pneumoniae	CWL-029	1/4
PC-E	Influenza A H3	A/Brisbane/10/07	1/4
	Parainfluenza virus Type 3	N/A	1/4
	Coronavirus 229E	N/A	1/4
	Mycoplasma pneumoniae	M129	1/4

Table. Performance of controls during Clinical trials

	PC-A	PC-B	PC-C	PC-D	PC-E	NC
easyMAG	9[a]/10	8[a]/9	6/6	11/11	8/8	50[b]/53
MP96	4/4	5/5	4/4	3/3	3/3	20/20

a - Operator error NC/PC switched during loading

b - Of 3 failed NCs, 1 was due to RNA IC failure, 1 was due to operator error (NC/PC switched on loading), 1 was due to detection of unexpected target (FP).

General Performance of Assay During Clinical Trials

Table. Accounting of valid and invalid runs during clinical trials (prospective specimens)

Description	Number	% of Total
Valid runs with complete results	57	86.4%
Completed Runs with NC failures	3	4.5%
Partially or completely invalid runs due to other failures	6	9.1%
Incomplete runs due to instrument failures	0	0.0%
Total	66	100%

Table. Summary of issues causing Invalid runs during clinical trials (prospective specimens)

Reason for failure	Number	% of Total
User Error	4	44.4%
Instrument/ Alignment[a]	2	22.2%
Negative control[b]	3	33.3%
Total invalid runs	9	100%

a – 2 consecutive failures for alignment error that did not happen again after adjustment.
b - Of 3 failed NCs, 1 was due to RNA IC failure, 1 was due to operator error (NC and PC switched on loading), 1 was due to detection of unexpected target (FP).

Analytical Performance

The results of the analytical studies summarized in the following paragraphs met the acceptance criteria and demonstrated the analytical performance characteristics of the proposed BioCode RPP.

Reproducibility Study

A study was performed to assess the Reproducibility of the BioCode® RPP on the BioCode® MDx-3000. Front end extraction was performed using the NucliSENS easyMAG and MagNA Pure 96 systems. This study was designed to assess intra-assay (within run), inter-assay (run-to-run), day-to-day and site-to-site reproducibility. One lot of reagents was assayed at 3 sites (2 sites with easyMAG and 1 site with MP96) by 2 operators on 1 instrument per site for 5 days (total of 10 runs per site). The reproducibility panel consisted of 6 contrived positive samples and 1 negative sample, each extracted in triplicate and each assayed in singlet. The samples consisted of combinations of 12 representative targets at 1.5x LoD (Low) and 3x LoD (Medium) spiked into simulated NPS in UTM matrix (see table below).

Table. Reproducibility panel

Medium (3x LoD)	**Medium (3x LoD)**	**Low (1.5x LoD)**	**Low (1.5x LoD)**	**Sample name**
Human Rhinovirus	Parainfluenza Virus 2	Human Metapneumovirus	*Bordetella pertussis*	RP1
Human Metapneumovirus	*Bordetella pertussis*	Human Rhinovirus	Parainfluenza Virus 2	RP2
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	Adenovirus C	RP5
Respiratory Syncytial Virus	Adenovirus C	Influenza A H3N2	*Mycoplasma pneumoniae*	RP6
Simulated Negative matrix				RP7

For each target, the results were determined according to the Interpretation Algorithm. Percent positive agreement was calculated for medium and low positives separately. Samples not containing said target were used to calculate percent negative agreement for each RPP target (see data tables below).

Conclusions: Acceptance criteria were met.

Results

Table. Results from the multi-site reproducibility study (viruses)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS easyMAG			MagNA Pure 96		All Sites
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	(95% CI)
Viruses								
Adenovirus	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**149/150** 99.30%	**299/300** 99.70%	**150/150** 100%	**150/150** 100%	**449/450** **99.80%** (98.8%-100%)
Coronavirus	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Human Metapneumovirus	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Human Rhinovirus/Enterovirus	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Influenza A/H3	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**29/30** [a] 96.70%	**30/30** 100%	**59/60** 98.30%	**30/30** 100%	**30/30** 100%	**89/90** **98.90%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**149/150** [b] 99.30%	**299/300** 99.70%	**150/150** 100%	**150/150** 100%	**449/450** **99.80%** (98.8%-100%)
Influenza A/H1pdm09	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Influenza A/H1	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Influenza B	**3× LoD**	**Detected**	**30/30**	**30/30**	**60/60**	**30/30**	**30/30**	**90/90**

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS easyMAG			MagNA Pure 96		All Sites
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	(95% CI)
			100%	100%	100%	100%	100%	**100%** (95.9%-100%)
	1.5× LoD	**Detected**	**29/30** 96.70%	**30/30** 100%	**59/60** 98.30%	**30/30** 100%	**30/30** 100%	**89/90** **98.90%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Parainfluenza Virus 1	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Parainfluenza Virus 2	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Parainfluenza Virus 4	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Parainfluenza Virus 3	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Respiratory Syncytial Virus	**3× LoD**	**Detected**	**29/30** 100%	**30/30** 100%	**59/60** 98.30%	**30/30** 100%	**30/30** 100%	**89/90** **98.90%** (94.0%-99.8%)
	1.5× LoD	**Detected**	**29/30** 100%	**30/30** 100%	**59/60** 98.30%	**30/30** 100%	**30/30** 100%	**89/90** **98.90%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)

a – There was an indeterminate Flu A result for Influenza A H3 low positive sample RP6

b – There was an indeterminate Flu A result for Influenza A H3 negative sample

Table. Results from the multi-site reproducibility study (bacteria)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS easyMAG			MagNA Pure 96		All Sites
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	(95% CI)
Bacteria								
Mycoplasma pneumoniae	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**149/150** 99.30%	**299/300** 99.70%	**150/150** 100%	**150/150** 100%	**449/450** **99.80%** (98.8%-100%)
Bordetella pertussis	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Chlamydia pneumoniae	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)

Multiple analyte spiked samples Vs single spiked samples

This study is to assess the performance of the BioCode® RPP on the BioCode® MDx-3000 with mixed analyte samples at or near the Limit of Detection (LoD) compared to single spiked samples to justify the use of multiple analyte spiked samples during analytical validation testing. The LoD was confirmed by extracting 20 replicates of each sample type and testing each in singlet for a total of 20 replicates at or near presumptive LoD. LoD for each stock was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20). The single spiked LoD was then challenged by pooling all 4 organisms and testing at 1x LoD (20 replicates). If 1x LoD for each analyte in the mixed pool does not meet the 95% detection goal (19/20), the organism was retested at 3x LoD. Results within 3x LoD was considered equivalent.

Results: All organisms were within 3x LoD for single and multiple spiked samples.

Table. Results from Multiple Vs Single spiked sample study stratified by extraction system.

Strain	Source	Target Probe	Single / Multiple Spike	Concentration	
				EasyMAG	MP96
Influenza A H3N2/A/Wisconsin/67/05a	Zeptometrix 0810252CF	FluA	Single Spike	1.3 $TCID_{50}$/mL	1.3 $TCID_{50}$/mL
		FluAH3			
		FluA	Multi Spike	4.0 $TCID_{50}$/mL	
		FluAH3			
Coronavirus NL63	Zeptometrix 0810228CF	NL63	Single Spike	0.013 $TCID_{50}$/mL	0.040 $TCID_{50}$/mL
			Multi Spike	0.040 $TCID_{50}$/mL	
Mycoplasma pneumoniae (M129)	Zeptometrix 0801579	MPN	Single Spike	5.0 CCU/mL	15.0 CCU/mL
			Multi Spike	15.0 CCU/mL	
Adenovirus C (type 2)	ATCC AV-846	ADV1	Single Spike	6.0 $TCID_{50}$/mL	18.0 $TCID_{50}$/mL
			Multi Spike		

Conclusion: Multiple spiked (mixed analyte) samples confirmed 95% LoD at the same or within 3-fold for each organism. Based on this testing multiple spiked samples may be used for other bench studies.

Limit of Detection (LoD)

A study was performed to assess the performance of the BioCode® RPP on the BioCode® MDx-3000 at the Limit of Detection (LoD) for specimens. In this study the BioCode® RPP was tested with quantified bacteria or viral stocks spiked in simulated NPS in UTM matrix. For initial screening, four replicates of each concentration were extracted on the easyMAG and MagNA Pure 96 Systems and tested in singlet with the BioCode® RPP on the BioCode® MDx-3000 system to estimate LoD. The LoD was confirmed by extracting 20 replicates of each sample type and testing each in singlet for a total of 20 replicates at or near the presumptive LoD. LoD for each stock was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20).

Results: See table below.

Table. Limit of Detection by extraction system

Target	Species/Strain/Isolate	Source	EasyMAG		MP96	
			Concentration	Detected (n of 20)	Concentration	Detected (n of 20)
Influenza A H1	A/New Caledonia/20/99	Zeptometrix 0810036CF	15.0 $TCID_{50}$/mL	20/20	5.0 $TCID_{50}$/mL	20/20
	A/NWS/33	ATCC VR-219	27.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	20/20
Influenza A H1 2009pdm	A(H1N1)/California/07/09	Zeptometrix 0810165CF	0.4 $TCID_{50}$/mL	20/20	0.4 $TCID_{50}$/mL	20/20
Influenza A H3	A/Wisconsin/67/05	Zeptometrix 0810252CF	4.0 $TCID_{50}$/mL	20/20	1.3 $TCID_{50}$/mL	20/20
	A/Alice	ATCC VR 776	27.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	19/20
Influenza B	Flu B/Florida/4/2006 (Yamagata)	Zeptometrix 0810255CF	0.01 $TCID_{50}$/mL	20/20	0.01 $TCID_{50}$/mL	20/20
	B/Hong Kong/S/1972 (Victoria)	ATCC VR-823	48.6 $TCID_{50}$/mL	20/20	48.6 $TCID_{50}$/mL	20/20
Respiratory Syncytial Virus	Type A	Zeptometrix 0810040ACF	0.33 $TCID_{50}$/mL	20/20	0.33 $TCID_{50}$/mL	20/20
Human Metapneumovirus	16; Type A1 IA10-2003	Zeptometrix 0810161CF	15.0 $TCID_{50}$/mL	19/20	15.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 1	C-35/Washington DC/1957	ATCC VR-94	9.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 2	Greer/Ohio/1955	ATCC VR-92	1.8 $TCID_{50}$/mL	20/20	5.4 $TCID_{50}$/mL	20/20
Parainfluenza Virus 3	N/A	Zeptometrix 0810016CF	15.0 $TCID_{50}$/mL	20/20	15.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 4	Type 4a	Zeptometrix 0810060CF	9.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	20/20
Adenovirus	Species B Serotype 7A	Zeptometrix 0810021CF	1.2 $TCID_{50}$/mL	20/20	1.2 $TCID_{50}$/mL	20/20
Adenovirus	Species C Serotype 2	ATCC VR-846	6.0 $TCID_{50}$/mL	20/20	18.0 $TCID_{50}$/mL	20/20
Adenovirus	Species E Serotype 4	Zeptometrix 0810070CF	0.04 $TCID_{50}$/mL	20/20	0.04 $TCID_{50}$/mL	19/20
Coronavirus 229E	N/A	Zeptometrix 0810229CF	0.6 $TCID_{50}$/mL	20/20	1.8 $TCID_{50}$/mL	20/20
Coronavirus HKU1	N/A	Clinical Sample 4922[a]	$5.02x10^4$ copies/mL	19/20	$5.02x10^4$ copies/mL	20/20
Coronavirus NL63	N/A	Zeptometrix 0810228CF	0.04 $TCID_{50}$/mL	20/20	0.04 $TCID_{50}$/mL	20/20
Coronavirus OC43	N/A	Zeptometrix 0810024CF	0.04 $TCID_{50}$/mL	20/20	0.01 $TCID_{50}$/mL	19/20
Rhinovirus	Type A1	Zeptometrix 0810012CF	1.2 $TCID_{50}$/mL	20/20	0.4 $TCID_{50}$/mL	19/20
Enterovirus	D68	Zeptometrix 0810300CF	3.0 $TCID_{50}$/mL	19/20	9.0 $TCID_{50}$/mL	20/20
Bordetella pertussis	A639	Zeptometrix 801459	15.0 CFU/mL	20/20	45.0 CFU/mL	19/20

Target	Species/Strain/Isolate	Source	EasyMAG		MP96	
			Concentration	Detected (n of 20)	Concentration	Detected (n of 20)
Chlamydia pneumoniae	AR39	ATCC VR-53592	16.7 CFU/mL	20/20	33.3 CFU/mL	20/20
Mycoplasma pneumoniae	M129	Zeptometrix 0801579	15.0 CCU/mL	20/20	15.0 CCU/mL	20/20

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

Conclusion: LoDs were comparable (within 3-fold) for each extraction system.

Analytical Reactivity/Inclusivity

A study was performed to verify Analytical Reactivity/Inclusivity of the BioCode® Respiratory Pathogen Panel (RPP). Different strains, serotypes and genotypes were selected that represent various temporal, geographic, and genetic diversity for each analyte. This study tested a panel of titered stocks of relevant organisms diluted in simulated NPS in UTM matrix starting at 3x LoD. Samples not detected at 3x LoD, were tested at higher concentrations. For Influenza B strains, all strains were grouped into Yamagata, Victoria or unknown lineages. Yamagata strains were tested according to the protocol at 3x and higher of the Yamagata strain LoD (LoD - 0.01 $TCID_{50}$/mL Flu B/Florida/4/2006). However, for Victoria strains, the validation LoD result was higher than expected (LoD-48.6 $TCID_{50}$/mL for Flu B /Hong Kong/S/1972) which could be related to difference in titration from vendor sources. Therefore, for Victoria strains, testing for inclusivity was performed starting at 0.03x and 0.3x of the Victoria strain LoD. Strains of unknown lineages of influenza B were tested at 3x LoD of Yamagata strain. Each sample was extracted in triplicate on the easyMAG and tested with the RPP on the BioCode® MDx-3000 system according to the instructions for use.

Assay reactivity for less common strains or serotypes that could not be tested due to unavailability was predicted using *in silico* analysis.

Results: The organisms listed below were detected at the concentrations indicated. See Table below.

Table. Influenza A isolates tested during the inclusivity study

Organism/ Type[a]	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Influenza A H1N1	Solomon Island/03/06	Zeptometrix	0810036CFN	45 $TCID_{50}$/mL	3x
	Singapore/63/04	Zeptometrix	0810246CF	45 $TCID_{50}$/mL	3x
	PR/8/34	Zeptometrix	0810245CF	45 $TCID_{50}$/mL	3x
	A/Brisbane/59/2007	Zeptometrix	0810244CF	45 $TCID_{50}$/mL	3x
	A/Taiwan/42/06	Zeptometrix	0810247CF	45 $TCID_{50}$/mL	3x
	A/New jersey/8/76[b]	ATCC	VR-897	$7.5x10^3$ $CEID_{50}$/mL	500x[b]

Organism/ Type[a]	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
	A/Denver/1/1957	VIRAPUR	NA	45 $TCID_{50}$/mL	3x
	A/FM/1/47	ATCC	VR-97	45 $CEID_{50}$/mL	3x
	A/Weiss/43[c]	ATCC	VR-96	$1.5x10^4$ $CEID_{50}$/mL	1000x[c]
	A/Beijing/262/95[d]	BEI	NR-12277	450 $CEID_{50}$/mL	30x[d]
	A/Mal/302/54	ATCC	VR-98	45 $CEID_{50}$/mL	3x
Influenza A H1N2	Recombinant; Kilbourne F64, A/NWS/1934 (HA) x A/Rockefeller Institute/ 5/1957 (NA)[e]	BEI	NR-3682	135 $CEID_{50}$/mL	5x[e]
Influenza A H1N1 pdm09	NY/01/09	Zeptometrix	0810248CF	1.2 $TCID_{50}$/mL	3x
	NY/02/09	Zeptometrix	0810109CFN	1.2 $TCID_{50}$/mL	3x
	NY/03/09	Zeptometrix	0810249CF	1.2 $TCID_{50}$/mL	3x
	A/Houston/3H/2009 (H1N1)pdm09[f]	BEI	NR-20340	1.2 $TCID_{50}$/mL	3x
	Influenza A H1N1pdm (Canada/6294/09)	Zeptometrix	0810109CFJ	1.2 $TCID_{50}$/mL	3x
	Influenza A H1N1pdm (Mexico/4108/09)	Zeptometrix	0810166CF	1.2 $TCID_{50}$/mL	3x
	California/04/09, cell isolate[g]	BEI	NR-13658	40 $TCID_{50}$/mL	100x[g]
	A/Christ Church/16/2010[h]	CDC	N/A	400 EID_{50}/mL	1000x[h]
	A/Brisbane/02/2018	CDC	N/A	40 EID_{50}/mL	100x[i]
Influenza A H3N2	A/Wisconsin/15/2009[j]	BEI	NR-42007	12 $CEID_{50}$/mL	3x
	A/Texas/50/12	Zeptometrix	0810238CF	12 $TCID_{50}$/mL	3x
	A/Brisbane/10/2007	Zeptometrix	0810138CF	12 $TCID_{50}$/mL	3x
	A/Port Chalmers/1/73	ATCC	VR-810	200 $CEID_{50}$/mL	50x[k]
	A/Victoria/3/1975	VIRAPUR	NA	12 $TCID_{50}$/mL	3x

Organism/ Type[a]	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
	A/Victoria/361/2011[l]	BEI	NR-44022	12 $CEID_{50}$/mL	3x
	A/Victoria/3/75	ATCC	VR-822	12 $CEID_{50}$/mL	3x
	A/Uruguay/716/07[m]	BEI	NR-42003	40 $TCID_{50}$/mL	10x
	A/HK/H090-756-V1(0)/2009[n]	BEI	NR-44344	12 $TCID_{50}$/mL	3x
	A/Hong Kong/8/68	Zeptometrix	0810250CF	12 $TCID_{50}$/mL	3x
	A/Switzerland/9715293/ 13	VIRAPUR	NA	12 $TCID_{50}$/mL	3x
	A/Aichi/2/68	ATCC	VR-547	12 $TCID_{50}$/mL	3x
	MRC-2	ATCC	VR-777	12 $TCID_{50}$/mL	3x
	A/Perth/16/2009	CDC	N/A	40 EID_{50}/mL	10x
	A/Kansas/14/2017[o]	CDC	N/A	8000 EID_{50}/mL	2000x[o]

a – *In silico* analysis predicts detection of Influenza A H2N3, H5N1, H5N2, H5N3, H5N8, H7N7, H7N9, H3N1, H3N2, H3N5, H3N7, H3N8 as Influenza A. However, predicted reactivities of the subtyping assays for these influenza A strains of animal origin are variable.

b – Influenza A H1N1 – [A/New jersey/8/76]. Detected as Flu A (no subtype) at 3x LoD. Detected as dual positive A/H1 and A/H1pdm09 at 500x LoD.

c – Influenza A H1N1 [A/Weiss/43]. Detected as Flu A (no subtype) at 100x LoD. *In silico* analysis showed several mismatches in the forward primer for the Flu A/H1 subtyping assay which may account for the observed lower sensitivity of the Flu A/H1 subtyping assay for this strain.

d – Influenza A H1N1 [A/Beijing/262/95]. Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Beijing/262/95 (H1N1), NR-12277. Detected as Flu A (no subtype) at 10x LoD. *In silico* analysis showed a G-A mismatch in the 3' terminal position in the forward primer for the Flu A/H1 subtyping assay which may account for the observed lower sensitivity of the Flu A/H1 subtyping assay for this strain.

e - Influenza A H1N2 [Recombinant]. Recombinant Virus obtained through BEI Resources, NIAID, NIH: Influenza A, Kilbourne F64, A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA)] (H1N2), NR-3682.Detected as Flu A (no subtype) at 3x LoD.

f – Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Houston/3H/2009 (H1N1)pdm09, NR-20340.

g – Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/California/04/09, cell isolate (H1N1) pdm09, NR-13658. *In silico* analysis of a partial sequence of this strain for the Flu A/H1pdm09 subtyping assay does not predict reduced analytical reactivity. Titering inconsistency from the vendor rather than reduced reactivity due to assay design is suggested.

h - Virus obtained through the CDC Influenza Division. *In silico* analysis of partial sequences of this strain for the Flu A/H1pdm09 HA subtyping assay and the Flu A matrix gene assay does not predict reduced analytical reactivity. Titering inconsistency from the source laboratory (EID_{50}/mL vs. $TCID_{50}$/mL) rather than reduced reactivity due to assay design is suggested.

i - Virus obtained through the CDC Influenza Division. *In silico* analysis did not reveal any critical mismatch in the Flu A/H1pdm09 HA subtyping assay primers and probe binding regions or any mismatch in the FluA matrix gene assay primers and probe binding regions that would predict reduced analytical reactivity. Titering inconsistency from the source laboratory (EID_{50}/mL vs. $TCID_{50}$/mL) rather than reduced reactivity due to assay design is suggested.

j – Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Wisconsin/15/2009 (H3N2), NR-42007.

k - *In silico* analysis revealed a few non-critical mismatches in the FluA/H3 subtyping HA assay probe binding region that could contribute to the observed lower reactivity. However, titering inconsistency from the vendor rather than reduced reactivity due to assay design is suspected.

l - Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Victoria/361/2011 (H3N2), NR-44022
m - Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Uruguay/716/07 (H3N2), NR-42003
n - Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/HK/H090-756-V1(0)/2009 (H3N2), NR-44344
o – Virus obtained through the CDC Influenza Division. This strain was detected by BioCode RPP as Flu A (no subtype) at 50xLoD. *In silico* analysis did not reveal any mismatch in the Flu A matrix assay primers and probe binding regions but showed 3 mismatches in the Flu A/H3 subtyping HA assay primers binding regions that could predict reduced analytical reactivity, 1 mismatch at the 9th position from the 3' end of the reverse primer (mismatch #1), 1 mismatch at the 18th position from the 3' end of the reverse primer (mismatch #2), and 1 mismatch at the 20th position from the 3' end of the reverse primer (mismatch #3). Wet testing data suggested that mismatch #1 is likely the root cause for the observed significant reduction in analytical reactivity for this strain. For patient samples contain a Flu A/H3 strain that harbors mismatch #1 at lower concentrations, the BioCode RPP will likely report a Flu A (no subtype detected) result. Although estimated prevalence of a sequence variant based solely on *in silico* analysis may not accurately reflect the actual prevalence of the sequence variant in circulation during an influenza season, based on an *in silico* analysis, of all the Flu A/H3 strains isolated in 2019 with published HA sequences, 73.8% of the strains harbor mismatch #1.

Table. Influenza B isolates tested during the inclusivity study

Organism/Lineage	Location/Strain/ Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[a]
Influenza B (Victoria[b])	B/Malaysia/2506/2004[d]	BEI	NR-9723	1.458 $TCID_{50}$/mL	0.03x
	B/Malaysia/2506/2004	Zeptometrix	0810258CF	1.458 $TCID_{50}$/mL	0.03x
	B/Ohio/01/2005[e]	BEI	NR-41801	14.58 $CEID_{50}$/mL	0.3x
	B/Brisbane/33/2008[f]	BEI	NR-42006	14.58 $CEID_{50}$/mL	0.3x
	B/Nevada/03/2011[g]	BEI	NR-44023	1.458 $CEID_{50}$/mL	0.03x
	B/Michigan/09/2011	CDC	N/A	14.58 EID_{50}/mL	0.3x
	B/Colorado/06/2017	CDC	N/A	14.58 EID_{50}/mL	0.3x
Influenza B (Yamagata)	B/Texas/06/2011[h]	BEI	NR-44024	50 $TCID_{50}$/mL	5000x[h]
	B/Sydney/507/2006[i]	BEI	NR-36526	8.0 $TCID_{50}$/mL	800x[i]
	B/Wisconsin/1/10	Zeptometrix	0810241CF	0.03 $TCID_{50}$/mL	3x
	B/Massachusetts/2/12	Zeptometrix	0810239CF	0.03 $TCID_{50}$/mL	3x
	B/Christchurch/33/2004[j]	BEI	NR-36536	0.03 $TCID_{50}$/mL	3x
	B/New Hampshire/01/2016[k]	CDC	N/A	10 EID_{50}/mL	1000x[k]
	B/Phuket/3073/2013[l]	CDC	N/A	5 EID_{50}/mL	500x[l]
	B/lee/1940	Zeptometrix	0810257CF	0.03 $TCID_{50}$/mL	3x

Organism/Lineage	Location/Strain/ Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[a]
Influenza B (unknown lineage[c])					
	B/Taiwan/2/1962	ATCC	VR-295	5.0 $CEID_{50}$/mL	500x[m]
	B/Allen/45	ATCC	VR-102	0.05 $CEID_{50}$/mL	5x
	B/Brigit	ATCC	VR-786	0.03 $TCID_{50}$/mL	3x

a - If either FluB assay has MFI above the cutoff, the software will report as Detected for Influenza B
b - For Victoria lineage strains testing started at 0.03x LoD rather than 3x LoD.
c - Strains of unknown lineages were assayed starting at 3x LoD of Yamagata strain.
d - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Malaysia/2506/2004, NR-9723.
e - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Ohio/01/2005, NR-41801.
f - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Brisbane/33/2008, NR-42006.
g - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Nevada/03/2011, NR-44023.
h - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Texas/06/2011, NR-44024. *In silico* analysis does not predict reduced analytical reactivity. Titering inconsistency from the vendor rather than reduced reactivity due to assay design is suggested.
i - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Sydney/507/2006, NR-36526. *In silico* analysis does not predict reduced analytical reactivity. Titering inconsistency from the vendor rather than reduced reactivity due to assay design is suggested.
j - Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Christchurch/33/2004, NR-36536.
k - Virus obtained through the CDC Influenza Division. *In silico* analysis did not reveal any critical mismatch in the Flu B NS1 assay primers and probe binding regions that would predict reduced analytical reactivity. And *In silico* analysis did not reveal any mismatch in the Flu B matrix assay primers and probe binding regions. Titering inconsistency from the source laboratory (EID_{50}/mL vs. $TCID_{50}$/mL) rather than reduced reactivity due to assay design is suggested.
l - Virus obtained through the CDC Influenza Division. *In silico* analysis did not reveal any critical mismatch in the Flu B NS1 assay primers and probe binding regions that would predict reduced analytical reactivity. And *In silico* analysis did not reveal any mismatch in the Flu B matrix assay primers and probe binding regions. Titering inconsistency from the source laboratory (EID_{50}/mL vs. $TCID_{50}$/mL) rather than reduced reactivity due to assay design is suggested.
m - *In silico* analysis could not be performed due to unavailability of sequence information for this strain. Titering inconsistency from the vendor rather than reduced reactivity due to assay design is suspected.

Table. Respiratory Syncytial Virus isolates tested during the inclusivity study

Organism/Type	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Respiratory Syncytial Virus Type A	TN/1998/3-2[a]	BEI	NR-28529	0.99 $TCID_{50}$/mL	3x
	TN/2000/3-4[b]	BEI	NR-28530	3.3 $TCID_{50}$/mL	10x
	TN/98/12-21[c]	BEI	NR-28528	0.99 $TCID_{50}$/mL	3x
	Long/Maryland/1956	ATCC	VR-26	3.3 $TCID_{50}$/mL	10x
Respiratory Syncytial Virus Type B	9320/Massachusetts/1977	ATCC	VR-955	0.99 $TCID_{50}$/mL	3x
	B1[d]	BEI	NR-4052	3.3 $TCID_{50}$/mL	10x
	WV/14617/85	ATCC	VR-1400	0.99 $TCID_{50}$/mL	3x
	18537/Washington DC/1962	ATCC	VR-1580	0.99 $TCID_{50}$/mL	3x
	CH93(18)18	Zeptometrix	0810040CF	0.99 $TCID_{50}$/mL	3x

a - Virus obtained through BEI Resources, NIAID, NIH: RSV, TN/1998/3-2, NR-28529
b - Virus obtained through BEI Resources, NIAID, NIH: RSV, TN/2000/3-4, NR-28530
c - Virus obtained through BEI Resources, NIAID, NIH: RSV, TN/98/12-21, NR-28528
d - Virus obtained through BEI Resources, NIAID, NIH: RSV, B1, NR-4052

Table. Human Metapneumovirus isolates tested during the inclusivity study

Genotype	Serotype	Location/ Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[a]
Human Metapneumovirus Type A1	9	Iowa3/2002	Zeptometrix	0810160CF	45 $TCID_{50}$/mL	3x
Human Metapneumovirus Type A2	27	Iowa27/2004	Zeptometrix	0810164CF	45 $TCID_{50}$/mL	3x
Human Metapneumovirus Type B1	3	Peru2/2002	Zeptometrix	0810156CF	45 $TCID_{50}$/mL	3x
	5	Peru3/2003	Zeptometrix	0810158CF	45 $TCID_{50}$/mL	3x
Human Metapneumovirus Type B2	4	Peru1/2002	Zeptometrix	0810157CF	45 $TCID_{50}$/mL	3x
	8	Peru6/2003	Zeptometrix	0810159CF	45 $TCID_{50}$/mL	3x
	18	Iowa18/2003	Zeptometrix	0810162CF	45 $TCID_{50}$/mL	3x
	Unknown	TN/91-316[b]	BEI	NR-22232	45 $TCID_{50}$/mL	3x

a - If either assay has MFI above the cutoff, the software will report as Detected for Human Metapneumovirus
b - Virus obtained through BEI Resources, NIAID, NIH: Human Metapneumovirus, TN/91-316, NR-22232

Table. Parainfluenza Virus (1-4) isolates tested during inclusivity.

Organism/Subtype	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Parainfluenza Virus 1	FRA/27344044/2007[a]	BEI	NR-48681	27 $TCID_{50}$/mL	3x
	FRA/29221106/2009[b]	BEI	NR-48680	27 $TCID_{50}$/mL	3x
	Unknown	Zeptometrix	0810014CF	27 $TCID_{50}$/mL	3x
Parainfluenza Virus 2	Greer[c]	BEI	NR-3229	5.4 $TCID_{50}$/mL	3x
	Unknown	Zeptometrix	0810015CF	5.4 $TCID_{50}$/mL	3x
Parainfluenza Virus 3	NIH 47885, Wash/47885/57[d]	BEI	NR-3233	45 $TCID_{50}$/mL	3x
	C243/Washington DC/1957	ATCC	VR-93	45 $TCID_{50}$/mL	3x
Parainfluenza Virus 4a	M-25/1958[e]	BEI	NR-3237	27 $TCID_{50}$/mL	3x
Parainfluenza Virus 4b	CH-19503/Washington DC/1962	ATCC	VR-1377	27 $TCID_{50}$/mL	3x
	Unknown	Zeptometrix	0810060BCF	27 $TCID_{50}$/mL	3x

a - Virus obtained through BEI Resources, NIAID, NIH: Parainfluenza Virus 1, HIPIV1/FRA/27344044/2007, NR-48681
b - Virus obtained through BEI Resources, NIAID, NIH: Parainfluenza Virus 1, HPIV1/FRA/29221106/2009, NR-48680
c - Virus obtained through BEI Resources, NIAID, NIH: Parainfluenza Virus 2, Greer, NR-3229
d - Virus obtained through BEI Resources, NIAID, NIH: Parainfluenza Virus 3, NIH 47885, NR-3233
e - Virus obtained through BEI Resources, NIAID, NIH: Parainfluenza Virus 4a, M-25, NR-3237

Table. Adenovirus isolates tested during the inclusivity study

Species[b]	Serotype	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[c]
Adenovirus A[a]	31	Unknown	Zeptometrix	0810073CF	18 $TCID_{50}$/mL	3x
	12	Huie/Massachusetts	ATCC	VR-863	18 $TCID_{50}$/mL	3x
	18	Washington DC/1954	ATCC	VR-19	18 $TCID_{50}$/mL	3x
Adenovirus B	3	GB/Maryland/1953	ATCC	VR-3	3.6 $TCID_{50}$/mL	3x
	14	Unknown	Zeptometrix	0810108CF	3.6 $TCID_{50}$/mL	3x
	16	CH.79/Saudi Arabia/1955	ATCC	VR-17	3.6 $TCID_{50}$/mL	3x
	35	Holden	ATCC	VR-718	3.6 $TCID_{50}$/mL	3x
Adenovirus C	1	Unknown	Zeptometrix	0810050CF	18 $TCID_{50}$/mL	3x
	5	Unknown	Zeptometrix	0810020CF	18 $TCID_{50}$/mL	3x

Species[b]	Serotype	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[c]
	6	Tonsil 99/Washington DC	ATCC	VR-6	18 $TCID_{50}$/mL	3x
Adenovirus D[a]	8	Unknown	Zeptometrix	0810069CF	18 $TCID_{50}$/mL	3x
	17	CH. 22/Saudi Arabia/1955	ATCC	VR-1836	18 $TCID_{50}$/mL	3x
	20	Unknown	Zeptometrix	0810115CF	18 $TCID_{50}$/mL	3x
	26	Unknown	Zeptometrix	0810117CF	18 $TCID_{50}$/mL	3x
	37	Unknown	Zeptometrix	0810119CF	18 $TCID_{50}$/mL	3x
Adenovirus F[a]	40	Unknown	Zeptometrix	0810084CF	18 $TCID_{50}$/mL	3x
	41	Tak/73-3544/Netherlands/1973	ATCC	VR-930	18 $TCID_{50}$/mL	3x

a – Adenovirus C LoD used as LoD was not determined for Species A, D or F.
b – *In silico* analysis predicts detection of Adenovirus species E serotypes (Adenovirus 4 strain was tested as a part of LoD studies as well.
c – If either AdV assay has MFI above the cutoff the software will report as Detected for Adenovirus.

Table. Coronavirus isolates tested during the inclusivity study

Organism	Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Coronavirus 229E	Unknown	ATCC	VR-740	1.8 $TCID_{50}$/mL	3x
Coronavirus NL63	Amsterdam/2003	BEI[b]	NR-470	0.12 $TCID_{50}$/mL	3x
Coronavirus OC43	Unknown	ATCC	VR-1558	0.12 $TCID_{50}$/mL	3x
HKU1[a]	Unknown	Clinical Sample 5016	Unknown	$1.51x10^5$ copies/mL	3x
	Unknown	Clinical Sample 5036	Unknown	$1.51x10^5$ copies/mL	3x
	Unknown	Clinical Sample 5037	Unknown	$5.02x10^5$ copies/mL	10x

a - Coronavirus HKU1 clinical samples titered with Applied BioCode validated SYBR assay using an IVT standard.
b - Virus obtained through BEI Resources, NIAID, NIH: Human Coronavirus NL63, NR-470

Table. Human Rhinovirus and Enterovirus isolates tested during the inclusivity study

Organism/ Species	Serotype/Strain/Isolate	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Rhinovirus A	Serotype 7/[68-CV11]	ATCC	VR-1601	3.6 $TCID_{50}$/mL	3x
	Serotype 16 [1A]	Zeptometrix	0810285CF	3.6 $TCID_{50}$/mL	3x
	Serotype 16 [11757/DC/1960]	ATCC	VR-283	3.6 $TCID_{50}$/mL	3x
	Serotype 34 [137-3]	ATCC	VR-1365	3.6 $TCID_{50}$/mL	3x
	Serotype 57 [Ch47}	ATCC	VR-1600	3.6 $TCID_{50}$/mL	3x
	Serotype 77 [130-63]	ATCC	VR-1187	3.6 $TCID_{50}$/mL	3x
	Serotype 80	Zeptometrix	0810288CF	3.6 $TCID_{50}$/mL	3x
	Serotype 85 [50-525-CV54]	ATCC	VR-1195	3.6 $TCID_{50}$/mL	3x
	Serotype 95 [SF-998}	ATCC	VR-1301	3.6 $TCID_{50}$/mL	3x
Rhinovirus B	Serotype 3 [FEB]	ATCC	VR-483	3.6 $TCID_{50}$/mL	3x
	Serotype 14	Zeptometrix	0810284CF	3.6 $TCID_{50}$/mL	3x
	Serotype 42	Zeptometrix	0810286CF	3.6 $TCID_{50}$/mL	3x
	Serotype 70	Zeptometrix	0810287CF	3.6 $TCID_{50}$/mL	3x
Enterovirus 71	EV 71	Zeptometrix	0810236CF	9.0 $TCID_{50}$/mL	3x

Table. *Bordetella pertussis* isolates tested during the inclusivity study

Organism	Strain/Isolate	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Bordetella pertussis	F	ATCC	8467	45 CFU/mL	3x
	5[17921]	ATCC	9340	45 CFU/mL	3x
	10-536	ATCC	10380	45 CFU/mL	3x
	CNCTC Hp 12/63[623]	ATCC	51445	45 CFU/mL	3x
	Tohama 1	ATCC	BAA-589	45 CFU/mL	3x
	MN2531	ATCC	BAA-1335	45 CFU/mL	3x

Table. *Mycoplasma pneumoniae* isolates tested during the inclusivity study

Organism	Strain/Isolate	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Mycoplasma pneumoniae	M129-B7	ATCC	29342	45 CFU/mL	3x
	PI 1428	ATCC	29085	45 CCU/mL	3x
	Mac	ATCC	15492	45 CFU/mL	3x
	UTMB-10P	ATCC	49894	45 CCU/mL	3x

Table. *Chlamydia pneumoniae* isolates tested during the inclusivity study

Organism	Strain/Isolate	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Chlamydia pneumoniae	CM-1	ATCC	VR-1360	50.1 CFU/mL	3x
	CWL-029	ATCC	VR-1310	50.1 CFU/mL	3x

Conclusion: This inclusivity testing suggests that the BioCode RPP is analytically reactive for the targeted organisms. BioCode RPP assay displayed good inclusivity for related organisms with similar genetic lineage/sequence.

Zoonotic Influenza A Analytical Reactivity/Inclusivity Testing

Due to public health concerns related to zoonotic transmission of influenza A viruses to humans (primarily swine and avian lineages), serial dilutions of the following isolates of swine and avian influenza A viruses (viral nucleic acids) were also tested assessing analytical reactivity:

- A/Japan/305/57 (H2N2)
- A/duck/Pennsylvania/10218/1984 (H5N2)
- A/turkey/Wisconsin/1/1966 (H9N2)
- A/Anhui/1/2013 (H7N9)
- A/Hubei/1/2010 (H5N1)
- A/Minnesota/11/2010 (H3N2v)

Consistent with the *in silico* predictions of analytical reactivity to zoonotic influenza A viruses, each non-seasonal influenza A strain tested (H2N2, H5N2, H9N2, H7N9, and H5N1), except for the H3N2v strain, was detected as Influenza A (no subtype) at various concentrations tested (i.e., detected by the BioCode RPP Influenza A matrix assay only). For the H3N2v strain, it was detected as Influenza A/H3 at higher concentrations but was detected as Influenza A (no subtype) at the lowest concentration tested.

Analytical testing could not be performed to assess reactivity to H5N8, H1N2v and H1N1 swine viruses due to the lack of availability of such zoonotic influenza A strains for wet testing. Based on an *in silico* analysis, H5N8 (KP739416), H1N2v (MK239077), and H1N1 swine (KP822962.1) were predicted to be detected as Influenza A (no subtype), Influenza A/H1 (with reduced sensitivity for the A/H1 assay), and Influenza A (no subtype), respectively.

Analytical Specificity/Cross Reactivity

A study was performed to verify that the BioCode® Respiratory Pathogen Panel (RPP) does not detect DNA or RNA from off-panel organisms commonly found in respiratory specimens or from organisms that can cause similar clinical symptoms. In addition, on-panel organisms were tested at high concentrations to ensure there is no cross-reactivity with other on-panel targets. This study tested a panel of titered stocks of relevant organisms. Organisms that were not available for wet testing were analyzed *in silico* comparing the whole organism sequence against all primers to assess potential for cross reactivity. Analysis was conducted using BLASTn and Primer-BLAST programs. All testing was performed at Applied BioCode®, Inc. Microorganisms were tested at 10^6 CFU/mL for bacteria or fungi and 10^5 $TCID_{50}$/mL for

virus or higher when possible. Stocks were combined with simulated NPS in UTM matrix at the time of extraction. Each organism was extracted in triplicate on the EasyMag and assayed in singlet with the RPP on the BioCode® MDx-3000 system according to the instructions for use. For each concentration tested, the number of replicates that gave valid results per the Interpretation Algorithm was determined. If any replicates were detected, testing was repeated from 5 additional extractions assayed in singlet. If detected after repeat with 5 additional replicates, serial dilutions were performed to determine the lower limit.

Table. Off-panel bacteria and fungi analyzed for analytical specificity (Cross reactivity)

Organism	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/N)
Acinetobacter baumannii	Zeptometrix	801597	9.67×10^6 CFU/mL	N
Aspergillus flavus	Zeptometrix	801598	1.72×10^6 CFU/mL	N
Bordetella bronchiseptica	Zeptometrix	801649	6.68×10^7 CFU/mL	N
Bordetella holmesii	Zeptometrix	801464	3.83×10^0 CFU/mL	Y[a]
Bordetella parapertussis	Zeptometrix	8011461	1.00×10^6 CFU/mL	N
Burkholderia cepacia	Zeptometrix	801584	4.13×10^7 CFU/mL	N
Candida albicans	Zeptometrix	801504	1.96×10^6 CFU/mL	N
Candida glabrata	Zeptometrix	801535	1.73×10^7 CFU/mL	N
Corynebacterium diphtheriae	Zeptometrix	801882	4.57×10^6 CFU/mL	N
Haemophilus influenzae	Zeptometrix	801679	2.40×10^6 CFU/mL	N
Klebsiella pneumoniae	Zeptometrix	801506	5.10×10^7 CFU/mL	N
Lactobacillus plantarum	Zeptometrix	801507	1.80×10^7 CFU/mL	N
Legionella longbeachae	Zeptometrix	8101577	1.93×10^7 CFU/mL	N
Legionella micdadei	Zeptometrix	801576	2.70×10^7 CFU/mL	N
Legionella pneumophila	Zeptometrix	801645	3.17×10^7 CFU/mL	N
Moraxella catarrhalis	Zeptometrix	801509	1.13×10^6 CFU/mL	N
Mycobacterium tuberculosis	Zeptometrix	801660	7.23×10^6 CFU/mL	N
Mycoplasma genitalium	ATCC	33530	1.00×10^3 CFU/mL	N
Mycoplasma hominis	ATCC	23114	2.7×10^4 CFU/mL	N
Neisseria elongata	Zeptometrix	801510	1.74×10^7 CFU/mL	N
Neisseria gonorrhoeae	Zeptometrix	801482	1.26×10^7 CFU/mL	N
Neisseria meningitidis	Zeptometrix	801511	2.55×10^6 CFU/mL	N
Neisseria sicca	Zeptometrix	801754	1.02×10^6 CFU/mL	N
Proteus vulgaris	Zeptometrix	801898	4.13×10^7 CFU/mL	N
Pseudomonas aeruginosa	ATCC	39324	2.11×10^6 CFU/mL	N
Serratia marcescens	Zeptometrix	801723	2.06×10^7 CFU/mL	N
Staphylococcus haemolyticus	Zeptometrix	801591	8.50×10^6 CFU/mL	N
Streptococcus agalactiae	Zeptometrix	801545	3.73×10^6 CFU/mL	N
Streptococcus dysgalactiae	Zeptometrix	801516	1.23×10^6 CFU/mL	N
Streptococcus intermedius	Zeptometrix	801895	5.07×10^6 CFU/mL	N
Streptococcus mitis	Zeptometrix	801695	5.73×10^6 CFU/mL	N
Streptococcus pneumoniae	Zeptometrix	801439	4.17×10^6 CFU/mL	N
Ureaplasma urealyticum	ATCC	27618	1.00×10^7 CFU/mL	N

a - *Bordetella holmesii* was detected by the *Bordetella pertussis* (BP) assay with 2 of 3 replicates down to 3.83 x 10^0 CFU/mL.

Table. Off-panel Viruses analyzed for analytical specificity (Cross reactivity)

Organism	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/N)
SARS-CoV, formaldehyde- and UV-inactivated, purified (vaccine)	BEI	NR-3883	1:100 Dilution	N[a]
MERS-CoV genomic RNA	BEI	NR-45843	1.01 x 10^7 Copies/mL	N
MERS-CoV EMC/2012, Heat-Inactivated	BEI	NR-50171	2 x 10^5 $TCID_{50}$/ml	N
Coxsackievirus A10	Zeptometrix	0810106CF	1.05 x 10^3 $TCID_{50}$/mL	Y[b]
Coxsackievirus A21	Zeptometrix	0810235CF	≤ 1.03 x 10^2 $TCID_{50}$/mL	Y[b]
Coxsackievirus A24	ATCC	VR-583	1.14 x 10^1 $TCID_{50}$/mL	Y[b]
Coxsackievirus B2	ATCC	VR-29	5.62 x 10^3 $TCID_{50}$/mL	Y[b]
Coxsackievirus B3	Zeptometrix	0810074CF	1.76 x 10^3 $TCID_{50}$/mL	Y[b]
Coxsackievirus B4	Zeptometrix	0810075CF	1.36 x 10^4 $TCID_{50}$/mL	Y[b]
Coxsackievirus B5	Zeptometrix	0810019CF	≤ 5.89 x 10^2 $TCID_{50}$/mL	Y[b]
Coxsackievirus A9	Zeptometrix	0810017CF	1.38 x 10^3 $TCID_{50}$/mL	Y[b]
Cytomegalovirus	Zeptometrix	0810003CF	4.17 x 10^4 $TCID_{50}$/mL	N
Echovirus 11	Zeptometrix	0810023CF	1.68 x 10^3 $TCID_{50}$/mL	Y[c]
Echovirus 30	Zeptometrix	0810078CF	≤ 1.95 x 10^2 $TCID_{50}$/mL	Y[c]
Echovirus 6	Zeptometrix	0810076CF	1.09 x 10^4 $TCID_{50}$/mL	Y[c]
Echovirus 9	Zeptometrix	0810077CF	1.07 x 10^1 $TCID_{50}$/mL	Y[c]
Epstein-Barr Virus	Zeptometrix	0810008CF	3.43 x 10^6 $TCID_{50}$/mL	N
Herpes Simplex Virus Type 1	Zeptometrix	0810187CF	9.12 x 10^6 $TCID_{50}$/mL	N
Measles Virus	Zeptometrix	0810025CF	1.31 x 10^5 $TCID_{50}$/mL	N
Mumps Virus	Zeptometrix	0810176CF	1.89 x 10^5 $TCID_{50}$/mL	N

a – Inhibitory (no RNA-IC detected) at 1:10 dilution

b – The Coxsackieviruses assayed here were detected by the Human Rhinovirus/Enterovirus (HRV) assay in at least 1 or the 3 replicates down to the concentrations indicated.

c – The Echoviruses assayed here were detected by the Human Rhinovirus/Enterovirus (HRV) assay in at least 1 or the 3 replicates down to the concentrations indicated.

Table. On-panel organisms analyzed for analytical specificity (Cross reactivity).

Organism	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/N)
Influenza A H1N1/New Caledonia/20/99	Zeptometrix	0810036CF	1.15 x 10^5 $TCID_{50}$/mL	N
Influenza A H1N1 /NWS/33	ATCC	VR-219	7.40 x 10^5 $TCID_{50}$/mL	N
Influenza A H1N1 pdm09/California/07/09	Zeptometrix	0810165CF	1.31 x 10^5 $TCID_{50}$/mL	N
Influenza A H3N2 /Wisconsin/67/05a	Zeptometrix	0810252CF	1.08 x 10^5 $TCID_{50}$/mL	N
Influenza A H3N2/Alice	ATCC	VR-776	1.43 x 10^6 $TCID_{50}$/mL	N
Influenza B/Florida/4/2006 (Yamagata)	Zeptometrix	0810255CF	1.08 x 10^5 $TCID_{50}$/mL	N
Influenza B/Hong Kong/S/1972 (Victoria)	ATCC	VR-823	8.57 x 10^5 $TCID_{50}$/mL	N
Respiratory Syncytial Virus (Type A)	Zeptometrix	0810040ACF	4.57 x 10^5 $TCID_{50}$/mL	N
Human Metapneumovirus 16 (Type A1)	Zeptometrix	0810161CF	8.51 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 1	ATCC	VR-94	1.60 x 10^5 $TCID_{50}$/mL	N

Organism	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/N)
Parainfluenza Virus 2	ATCC	VR-92	1.35 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 3	Zeptometrix	0810016CF	3.39 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 4a	Zeptometrix	0810060CF	1.13 x 10^5 $TCID_{50}$/mL	N
Adenovirus Species B Serotype 7A	Zeptometrix	0810021CF	5.83 x 10^5 $TCID_{50}$/mL	N
Adenovirus Species C Serotype 2	ATCC	AV-846	2.81 x 10^5 $TCID_{50}$/mL	N
Adenovirus Species E Serotype 4	Zeptometrix	0810070CF	1.08 x 10^5 $TCID_{50}$/mL	N
Coronavirus 229E	Zeptometrix	0810229CF	1.09 x 10^5 $TCID_{50}$/mL	N
Coronavirus HKU1	N/A	Clinical sample[a]	1.92 x 10^5 $TCID_{50}$/mL	N
Coronavirus NL63	Zeptometrix	0810228CF	1.08 x 10^5 $TCID_{50}$/mL	N
Coronavirus OC43	Zeptometrix	0810024CF	1.08 x 10^5 $TCID_{50}$/mL	N
Human Rhinovirus Type A1	Zeptometrix	0810012CF	1.05 x 10^5 $TCID_{50}$/mL	N
Enterovirus D68	Zeptometrix	0810300CF	1.08 x 10^5 $TCID_{50}$/mL	N
Bordetella pertussis	Zeptometrix	801459	3.86 x 10^7 CFU/mL	N
Mycoplasma pneumoniae	Zeptometrix	801579	1.06 x 10^6 CCU/mL	N
Chlamydia pneumoniae (AR-39)	ATCC	53592	1.24 x 10^6 CFU/mL	N
Chlamydia pneumoniae (CWL-029)	ATCC	VR-1310	1.00 x 10^6 CFU/mL	N

a - Coronavirus HKU1 clinical samples titered with Applied BioCode validated SYBR assay using an IVT RNA standard.

Conclusion: Cross reactivity was not observed with the on-panel or off-panel microorganisms at the concentrations tested in this study except the following:

- Empirical testing and *in silico* sequence analysis indicate that the Rhinovirus/Enterovirus assay (HRV) may also react with other Enterovirus species (i.e., Coxsackievirus and Echoviruses; see table for testing results).
- *In silico* sequence analysis indicate that the *Bordetella pertussis* assay (BP) may react with *Bordetella holmesii* and *Bordetella bronchiseptica*.

Interfering Substances/Microbes

A study was performed to demonstrate the accuracy of the BioCode® Respiratory Pathogen Panel on the BioCode® MDx-3000 in the presence of potentially interfering substances or microorganisms. Each member of the interfering substance panel was added to contrived samples in simulated NPS (sNPS) in UTM matrix containing representative members of the BioCode RPP at 3x LoD and a negative sample comprised of only sNPS in UTM matrix. Each sample was tested with and without potentially interfering substances. Each sample was extracted in triplicate on both the easyMAG and MP96 extraction systems and tested singly with the RPP on the BioCode® MDx-3000 system. Concentrations of interferents were determined by reviewing results of previous RP clinical trials. Substances that produce interference at the original test concentration were tested at lower concentrations.

Table . Contrived samples (3x LoD in sNPS)

Sample Name	Organism	Source
RPP A	Adenovirus B Serotype 7A	Zeptometrix 0810060CF
	Mycoplasma pneumoniae	Zeptometrix 801579

	Influenza A H3N2 A/Wisconsin/67/2005	Zeptometrix 0810252CF
RPP B	Respiratory Syncytial Virus (Type A)	Zeptometrix 0810040ACF
	Influenza A H1N1/California/07/09	Zeptometrix 0810165CF
	Human Metapneumovirus (16; type A1)	Zeptometrix 0810161CF
RPP C	Parainfluenza Virus 3	Zeptometrix 0810016CF
	Coronavirus NL63	Zeptometrix 0810228CF
	Influenza B/Florida/4/2006	Zeptometrix 0810255CF
HRV	Human Rhinovirus	Zeptometrix 0810012CF

Results: All targets of Samples RPP A, RPP B, RPP C and HRV were detected (3/3) at the concentrations below, suggesting no interference from these potential interferents at the concentrations tested.

Table: Evaluation for microbial interferents on BioCode® RPP

Microbial Interferent	Brand/Source	Concentration	Interference Yes (Y) or No (N)
Streptococcus pneumoniae	Zeptometrix	1 X 10^6 CFU/mL	N
Haemophilus influenzae	Zeptometrix	1 X 10^6 CFU/mL	N
Neisseria meningitidis	Zeptometrix	1 X 10^6 CFU/mL	N
Staphylococcus aureus	ATCC	1 X 10^6 CFU/mL	N
Cytomegalovirus	Zeptometrix	1 X 10^5 $TCID_{50}$/mL	N

Table: Non-microbial Interfering substances tested for BioCode® RPP assay

Substance Interferent	Brand/Source	Concentration	Interference Yes (Y) or No (N)
Genomic DNA	Promega	10 ng/µl	N
Mucin (MagNA Pure 96)	Sigma	0.6% W/V	N
Mucin (EasyMAG)[a]	Sigma	0.5% W/V	N
Human Blood	Poplar Health	1% V/V	N
Zanamivir	APExBIO	550 ng/mL	N
Oseltamivir	APExBIO	142 ng/mL	N
Nasal spray	Equate	1% V/V	N
Nasal decongestant spray	Bayer	1% V/V	N
Nasal Allergy spray (Fluticasone)	Equate	1.5% V/V	N
Petroleum Jelly	Equate	1% W/V	N
Analgesic Ointment	Vicks	1% W/V	N
Mupirocin	Alfa Aesar™	2% W/V	N
Tobramycin	MP Biomedicals,LLC	0.6 mg/mL	N
Bleach (10%)	VWR	5% V/V	N
Disinfecting wipes	Clorox	50% V/V	N
Ethanol (70%)	LabChem	7% V/V	N
Remel M4 Media	Remel	90% V/V	N
Remel M4-RT Media	Remel	90% V/V	N
Remel M5 Media	Remel	90% V/V	N
Remel M6 Media	Remel	90% V/V	N
Copan FloQ (Flocked nylon/plastic shaft)	Copan	1 swab	N

Substance Interferent	Brand/Source	Concentration	Interference Yes (Y) or No (N)
Copan 168C (rayon/twisted aluminum shaft)	Copan	1 swab	N
Polyester / Aluminum shaft swab	Puritan/Copan	1 swab	N
DNAzap	Invitrogen	1% V/V	N
RnaseOut	Invitrogen	1% V/V	N

a - It was observed that mucin at higher concentration (0.6%) led to loss of signal for some targets (loss of analyte detection) when extracted with NucliSENS easyMAG.

Table: Nasal influenza vaccine (FluMist) tested for BioCode® RPP assay

FluMist® 2010-2011 (V/V%)	Influenza A			Influenza B
	H1	H1N1-2009	H3	
10%	-	+	+	+
1%	-	+	+	+
0.1%	-	+	+	+
0.01%	-	+	+	+
0.001%	-	+	+	+
0.0001%	-	+	+[a]	+
0.00001%	-	-	-	+[a]
0.000001%	-	-	-	-

a - 2/3 replicates detected

Conclusion: None of the substances were shown to interfere with BioCode® RPP at the concentrations tested. However, it was observed that mucin at higher concentration (0.6%) could lead to loss of signal for some targets (loss of analyte detection) when extracted with easyMAG. The effect of mucin was dependent on the concentration in the sample tested.

Flu Mist was evaluated to be reactive as predicted with the BioCode RPP assay, therefore recent administration or contamination of specimens by Flu vaccine prior to NPS collection could lead to false detection by BioCode® RPP.

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 ($\geq 10^6$ CFU/mL for bacteria and $\geq 10^5$ units/mL for viruses) and two targets at low concentration ($\leq$3x LoD). Common co-infections were determined by reviewing results of previous Respiratory Panel clinical trials from 510k summaries, publications/posters and internal clinical sample testing. Each sample was extracted in triplicate on the easyMAG and each extraction tested in singlet with the RPP on the BioCode® MDx-3000 system.

Results: Results are shown in the table below.

Table. Competitive inhibition testing results

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Result (n of 3 Detected)
Competitive Inhibition Sample 1	Adenovirus species C Serotype 2	ATCC VR-846	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Respiratory syncytial virus Type A	Zeptometrix 0810040ACF	Low	0.99 $TCID_{50}$/mL	3/3
	Influenza A H3N2 A/Wisconsin/67/05a	Zeptometrix 0810252CF	Low	12 $TCID_{50}$/mL	3/3
					3/3
Competitive Inhibition Sample 2	Respiratory syncytial virus Type A	Zeptometrix 0810040ACF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza A H3N2 A/Wisconsin/67/05a	Zeptometrix 0810252CF	Low	12 $TCID_{50}$/mL	3/3
					3/3
	Adenovirus species C Serotype 2	ATCC VR-846	Low	18 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 3	Influenza A H3N2 A/Wisconsin/67/05a	Zeptometrix 0810252CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
					3/3
	Adenovirus species C Serotype 2	ATCC VR-846	Low	18 $TCID_{50}$/mL	3/3
	Respiratory syncytial virus Type A	Zeptometrix 0810040ACF	Low	0.99 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 4	Coronavirus OC43	Zeptometrix 0810024CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Human Metapneumovirus	Zeptometrix 0810161CF	Low	45 $TCID_{50}$/mL	3/3
	Bordetella pertussis	Zeptometrix 801459	Low	45 CFU/mL	3/3
Competitive Inhibition Sample 5	Human Metapneumovirus	Zeptometrix 0810161CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Bordetella pertussis	Zeptometrix 801459	Low	45 CFU/mL	3/3
	Coronavirus OC43	Zeptometrix 0810024CF	Low	0.12 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 6	*Bordetella pertussis*	Zeptometrix 801459	High	$1x10^6$ CFU/mL	3/3
	Coronavirus OC43	Zeptometrix 0810024CF	Low	0.12 $TCID_{50}$/mL	3/3
	Human Metapneumovirus	Zeptometrix 0810161CF	Low	45 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 7	Influenza A H1N1 pdm California/07/09	Zeptometrix 0810165CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
					3/3
	Parainfluenza Virus 3	Zeptometrix 0810016CF	Low	45 $TCID_{50}$/mL	3/3
	Human Rhinovirus type A	Zeptometrix 0810012CFN	Low	3.6 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 8	Parainfluenza Virus 3	Zeptometrix 0810016CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Human Rhinovirus type A	Zeptometrix 0810012CFN	Low	3.6 $TCID_{50}$/mL	3/3

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Result (n of 3 Detected)
	Influenza A H1N1 pdm California/07/09	Zeptometrix 0810165CF	Low	1.2 $TCID_{50}$/mL	3/3
					3/3
Competitive Inhibition Sample 9	Human Rhinovirus type A	Zeptometrix 0810012CFN	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza A H1N1 pdm California/07/09	Zeptometrix 0810165CF	Low	1.2 $TCID_{50}$/mL	3/3
					3/3
	Parainfluenza Virus 3	Zeptometrix 0810016CF	Low	45 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 10	*Mycoplasma pneumoniae*	Zeptometrix 801579	High	$1x10^6$ CCU/mL	3/3
	Coronavirus NL63	Zeptometrix 0810228CF	Low	1.2 $TCID_{50}$/mL	3/3
	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	Low	0.04 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 11	Coronavirus NL63	Zeptometrix 0810228CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	Low	0.04 $TCID_{50}$/mL	3/3
	Mycoplasma pneumoniae	Zeptometrix 801579	Low	45 CCU/mL	3/3
Competitive Inhibition Sample 12	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Mycoplasma pneumoniae	Zeptometrix 801579	Low	45 CCU/mL	3/3
	Coronavirus NL63	Zeptometrix 0810228CF	Low	1.2 $TCID_{50}$/mL	3/3

Conclusion: All replicates from each pooled sample were valid and detected. No competitive inhibition was observed at the concentrations tested.

Cross Contamination/Sample Carryover

Carry-over contamination studies have been performed for the BioCode® MDx-3000 system in conjunction with the easyMAG (K180041) and MagNA Pure 96 systems (K190585). Since this study is not assay-specific, no additional testing was performed for BioCode® RPP.

Specimen Stability

A study was performed to assess the Specimen Stability limitations for the optimal performance of the BioCode® Respiratory Pathogen Panel (RPP) on the BioCode® MDx-3000. Representative organisms from the BioCode® RPP were spiked into prescreened negative natural NPS in UTM matrix at 3x LoD and assayed with 3 extractions on the easyMAG at each timepoint (7 at timepoint 0). This study assessed the following storage conditions:

Table. Specimen stability conditions

Nasopharyngeal Swabs in transport media		
Storage temp	Target (storage time)	Test time points
Room Temperature 25ºC	8 hours	8,12 hours
Refrigerated 4ºC	7 days	5, 7, 10 days
Fresh Vs Frozen (-70ºC)	90 days (2x freeze/thaw)	30, 60, 90 days
Purified nucleic acids		
Storage temp	Target (storage time)	Test time points
Refrigerated 4ºC	8 hours	8, 12 hours
Fresh Vs Frozen (-70ºC)	90 days (2x freeze/thaw)	30, 60, 90 days

Table. Contrived samples (3x LoD in prescreened negative natural matrix)

Sample Name	Organism	Source
RP A	Adenovirus B Serotype 7a	Zeptometrix 0810021CF
	Mycoplasma pneumoniae	Zeptometrix 0801579
	Influenza A H3N2 A/Wisconsin/67/2005	Zeptometrix 0810252CF
RP B	Respiratory Syncytial Virus (Type A)	Zeptometrix 0810040ACF
	H1N1pdm California/07/09	Zeptometrix 0810165CF
	Human Metapneumovirus (16; type A1)	Zeptometrix 0810161CF
RP C	Parainfluenza Virus 3	Zeptometrix 0810016CF
	Coronavirus NL63	Zeptometrix 0810228CF
	Flu B/Florida/4/2006	Zeptometrix 0810255CF

Results: All replicates were Valid and detected for all conditions assayed. Summary results are shown in the table below.

Table. Qualitative results from Specimen Stability study

			Sample A			Sample B			Sample C		
Sample Type	**Temp**	**Time (Hours or Days)**	**ADV**	**MPN**	**Flu A H3N2**	**RSV**	**Flu A H1N1 2009 pdm**	**hMPV**	**PIV3**	**NL63**	**Flu B**
Fresh (baseline)	N/A	0	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7
Spiked NPS	Room Temp	8 Hour	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		12 Hour	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	4°C	5 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		7 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		10 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	-80°C	30 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		60 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		90 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Extracted Nucleic acid	4°C	8 Hour	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		12 Hour	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	-80°C (2x Freeze/thaw)	30 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		60 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
		90 Day	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

Conclusions: All replicates were Valid and detected for each specimen stability condition.

Matrix equivalency: Simulated Nasopharyngeal Swabs (sNPS) vs Native Nasopharyngeal Swabs (NPS)

Analytical validation studies are mostly performed with contrived specimens (i.e., spiking known concentrations of pathogens into negative NPS in UTM or VTM matrix). This would require a large volume of negative natural NPS to be collected and screened, while typically only between 1.0 to 2.0 mL per donor could be obtained after NPS collection and screening to confirm negative status. This makes collection from donors burdensome. An analytical study was performed to assess the performance equivalency of testing a simulated NPS (sNPS) in UTM matrix compared to testing natural NPS in UTM using the BioCode RPP, with both the NucliSENS easyMAG and the MagNA Pure 96 extraction systems. The sNPS in UTM matrix consists of $2x10^3$ HeLa cells/mL diluted in UTM.

Samples were contrived in negative natural NPS in UTM matrix and in sNPS in UTM matrix with multi-spiked pathogens at close to LoD levels (approximately 1.5x to 15.0xLoD). Each sample was extracted in quadruplicate on both the easyMAG and the MagNA Pure 96 extraction system and tested singly with the BioCode RPP on the BioCode MDx-3000 system.

Results: All four replicates of pathogens in three pools were Valid and detected. No unexpected targets were detected in the sNPS.

Table. Results of natural NPS Vs sNPS comparison

Pools	Organism	Source	Concentration	Multiple of LoD tested		Detected (N of 4)			
				easyMAG	MagNA Pure 96	easyMAG		MagNA Pure 96	
						Natural NPS	sNPS	Natural NPS	sNPS
Pool A	Adenovirus E Serotype 4	Zeptometrix 0810070CF	0.6 $TCID_{50}$/mL	15x LoD	15x LoD	4/4	4/4	4/4	4/4
	Chlamydia pneumoniae	ATCC 53592	75.0 CFU/mL	4.5x LoD	2.3x LoD	4/4	4/4	4/4	4/4
	Influenza A H3N2 A/Wisconsin/67/2005	Zeptometrix 0810252CF	6.0 $TCID_{50}$/mL	1.5x LoD	4.6x LoD	4/4	4/4	4/4	4/4
Pool B	Influenza A H1N1/New Caledonia/ 20/99	Zeptometrix 0810036CF	67.5 $TCID_{50}$/mL	4.5x LoD	13.5x LoD	4/4	4/4	4/4	4/4
	Respiratory Syncytial Virus (Type A)	Zeptometrix 0810040ACF	1.5 $TCID_{50}$/mL	4.5x LoD	4.5x LoD	4/4	4/4	4/4	4/4
	Human Metapneumovirus (16; type A1)	Zeptometrix 0810161CF	67.5 $TCID_{50}$/mL	4.5x LoD	4.5x LoD	4/4	4/4	4/4	4/4
Pool C	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	0.06 $TCID_{50}$/mL	6x LoD	6x LoD	4/4	4/4	4/4	4/4
	Coronavirus OC43	Zeptometrix 0810024CF	0.06 $TCID_{50}$/mL	1.5x LoD	6x LoD	4/4	4/4	4/4	4/4
	Parainfluenza Virus 4	Zeptometrix 0810060CF	13.5 $TCID_{50}$/mL	1.5x LoD	1.5x LoD	4/4	4/4	4/4	4/4
Negative Control		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Conclusions: No matrix effect was observed in this study. All replicates for each contrived sample assayed with the simulated matrix (sNPS- HeLa cells in UTM) and natural matrix were valid and detected for expected targets per the algorithm using both extraction systems easyMAG and MagNA Pure 96 extraction systems.