Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

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

I Background Information:

A 510(k) Number

K192485

B Applicant

Applied BioCode, Inc.

C Proprietary and Established Names

BioCode Respiratory Pathogen Panel (RPP)

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:
New device

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

C Type of Test:
A multiplexed nucleic acid test intended for use with the BioCode MDx-3000 Instrument for the simultaneous qualitative *in vitro* detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) collected in viral transport media (VTM) or universal transport media (UTM), and obtained from individuals suspected of respiratory tract infections.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

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

- Adenovirus
- Coronavirus (229E, OC43, HKU1, and NL63)
- Human Metapneumovirus A/B
- Influenza A, including subtypes H1, H1 2009 Pandemic, and H3
- Influenza B
- Parainfluenza 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.

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

D Special Instrument Requirements:
BioCode MDx-3000 Instrument
bioMérieux NucliSENS easyMAG system
Roche MagNA Pure 96 system

IV Device/System Characteristics:

A Device Description:
The BioCode Respiratory Pathogen Panel (RPP) is a respiratory pathogen multiplex nucleic acid test designed for use with the BioCode MDx-3000 system. The BioCode MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for multiple viral and bacterial pathogens from a single nasopharyngeal swab specimen collected in VTM or UTM. Liquid specimens are processed, and nucleic acids extracted with the bioMérieux NucliSENS easyMAG or Roche MagNA Pure 96 automated systems. Once the PCR plate is manually set up and sealed, all other operations are automated on the MDx-3000. The BioCode RPP simultaneously tests for 17 pathogens and/or subtypes (see the Intended Use section above) from nasopharyngeal swab specimens collected in VTM or UTM. Results from the BioCode RPP test are available within about 5 hours.

Materials Provided with Each BioCode RPP Kit:
- BioCode Master Mix A
- BioCode RPP Primer Mix
- BioCode RT Mix

- BioCode RNA IC2
- BioCode RPP BMB-Probe Mix
- Individually-packaged Transfer Pipettes

Materials Needed but Not Provided with the BioCode RPP Kit:

- BioCode SA-PE Mix
- BioCode Buffer A10% bleach solution
- BioCode MDx-3000 Instrument
- BioCode MDx-3000 Consumables:
    - Reagent Reservoirs (Integra 4332)
    - Waste Bin and Lid (Applied BioCode 01-W0105)
    - 20 µL pipette tips (Beckman 717256)
    - 250 µL pipette tips (Beckman 717252)
    - 96-well hard shell plate 0.1 mL (Bio-Rad HSL9601)
    - PCR Adhesive Foil (Thermo Fisher Scientific AB-0626)
    - Microtiter plate (Greiner bio-one 655101)
    - Microtiter plate lid (Nunc 5500)
- NucliSENS easyMAG (bioMérieux) Extraction System
- NucliSENS easyMAG (bioMérieux) Supplies for Extraction:
    - Lysis Buffer
    - Buffer 1
    - Buffer 2
    - Buffer 3
    - Magnetic silica
    - Nuclease free water
    - Consumables
    - ELISA strip plate

or

- MagNA Pure 96 (Roche) Extraction System
- MagNA Pure 96 (Roche) Supplies for Extraction:
    - MagNA Pure 96 DNA and Viral nucleic acid kit
    - MagNA Pure 96 system fluid
    - Consumables
- Vortex
- Centrifuge
- Pipettes – single, multi-channel and/or repeater with accuracy range between 1-10 µL, 10-200 µL, and 100 – 1000 µL
- Sterile, RNase/DNase-free disposable aerosol-barrier micro pipettor tips
- 1.5 mL polypropylene micro centrifuge tubes and racks (RNase/DNase free recommended)
- Cooler racks for 1.5 mL tubes and 0.1 mL 96 well plate
- Biosafety cabinet (laminar flow hood) for nucleic acids extractions
- Freezer (manual defrost) at -10°C to -30°C
- Freezer (manual defrost) at -60°C to -90°C
- Refrigerator at 2 to 8°C

Interpretation of Results
The BioCode MDx-3000 software will analyze data based on plate validity, sample validity and Median Fluorescent Intensity (MFI) compared to an MFI threshold.

Run Controls and Internal Control Results
The BioCode MDx-3000 software will suppress results if Internal or Negative controls are invalid. The software will indicate if external positive controls are valid or invalid but will not suppress results if the positive control is not valid (see *Internal Control*) below.

External Negative Controls
External Negative Controls can be RNase-free water, transport media, or well characterized negative specimens. The External Negative Controls should go through all processing steps (extractions, amplification, and detection). At least one External Negative Control is required for each plate/kit lot. The BioCode MDx-3000 software will suppress results for all samples if the External Negative Control is not valid (see Table 1 below).

Table 1: Criteria for Valid External Negative Control

Control	Targets	RNA IC	Description
Negative Control	Not Detected	Detected	Plate Status: Valid. Samples can be interpreted.
Negative Control	Detected	N/A	Plate Status: Invalid. Samples results cannot be interpreted. Results suppressed by software.
Negative Control	N/A	Not Detected	Plate Status: Invalid. Samples results cannot be interpreted. Results suppressed by software.

External Positive Controls
External Positive Controls can be well characterized clinical samples or positive strains. External Positive Controls can be single analytes or pooled multianalyte specimens. External Positive Controls should go through all processing steps (extractions, amplification, and detection). It is recommended that at least one External Positive Control be included for each plate/kit lot on a rotating schedule. Wells identified as External Positive Controls will be trended by the BioCode MDx-3000 software and the report will indicate a valid or invalid result on the report header (see Table 2 below). However, the software will not suppress results based on External Positive Control results. If an External Positive Control does not perform as expected, the user should review all samples in that plate to determine if results can be reported.

Table 2: Criteria for Valid External Positive Control

Control	Targets	RNA IC	Recommendations
Positive Control	Expected Target Detected	N/A	Report will indicate Positive Control is Valid. No user intervention required.
Positive Control	Expected Target Not Detected	N/A	Report will indicate Positive Control is Invalid. User should review results of all samples in that plate prior to release.
Positive Control	Unexpected Target Detected	N/A	Report will indicate Positive Control is Invalid. User should review results of all samples in that plate prior to release.

Internal Control

An RNA Internal Control (RNA IC: bacteriophage MS2) is added to each sample during extraction. The Internal Control monitors the efficiency of the extraction, reverse transcription, amplification and detection stages of the assay. Positive target assay results may be reported in the absence of RNA IC detection. However, the BioCode MDx-3000 software will suppress target assay negative results for any wells with invalid RNA IC results (see Table 3 below). Lack of RNA IC signal may indicate sample-associated inhibition or reagent/instrumentation issues. Samples suspected of being inhibitory should be repeated from the extraction step. If reagent or instrument issues are suspected specimens may be repeated using residue nucleic acid extracts.

Table 3: Criteria for RNA Internal Control (RNA IC)

Targets	RNA IC	Recommendations
N/A	Detected	Well status: Valid. Report all results.
Detected	Not Detected	Well status: Invalid. Detected results may be reported. Consider repeat/reflex testing.
Not Detected	Not Detected	Well status: Invalid. Not Detected results suppressed by software. Repeat/reflex testing.

Interpretation of Pathogen Results

Fluorescent signals from barcoded magnetic beads (BMBs) with the same barcode are sorted and the median fluorescence index (MFI) is calculated for each analyte assay. The assays are considered "Detected" by comparing the MFI to a specific validated assay cutoff. If assay target specific MFI is at or above the threshold, the assay is positive.

For the following organisms detected by the BioCode RPP, the organism is reported as "Detected" if a single corresponding assay is positive.

- Rhinovirus/Enterovirus
- Parainfluenza Virus 1
- Parainfluenza Virus 2
- Parainfluenza Virus 3
- Parainfluenza Virus 4
- Respiratory Syncytial Virus A/B
- *Bordetella pertussis*

- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

For the following organisms detected by the BioCode RPP, the organism is reported as "Detected" if at least one of the two corresponding assays is positive.

- Influenza B
- Human Metapneumovirus A/B
- Adenovirus

And for the following organism detected by the BioCode RPP, the organism is reported as "Detected" if at least one of the four corresponding assays is positive.

- Coronavirus

Note: Each of the four Coronavirus (CoV) assays is specifically designed to detect CoV-229E, CoV-OC43, CoV-HKU1, and CoV-NL63, respectively.

In addition, the BioCode RPP contains one assay designed to detect Influenza A (FluA), and three hemagglutinin (HA) subtyping assays for A/H1, A/H1pdm09 and A/H3, respectively. Each of the individual assays is interpreted independently and the panel result reported for Influenza A is based on the combined results of the four assays as outlined in Table 4 below.

Table 4: Possible Target Assay Results for Influenza A and Corresponding Interpretation

Target Assay / BioCode RPP Result	FluA	A/H1	A/H1pdm09	A/H3	Action
Influenza A Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Report result
Influenza A and A/H1 Detected	Detected	Detected	Not Detected	Not Detected	Report result
Influenza A and A/H3 Detected	Detected	Not Detected	Not Detected	Detected	Report result
Influenza A and A/H1pdm09 Detected	Detected	Not Detected	Detected	Not Detected	Report result
Influenza A/H1, and A/H3 Detected	Detected	Detected	Not Detected	Detected	Multiple infections are possible but rare, retest to confirm result [a]
Influenza A/H1pdm09, and A/H3 Detected	Detected	Not Detected	Detected	Detected	Multiple infections are possible but rare, retest to confirm result [a]
Influenza A/H1, and A/H1pdm09 Detected	Detected	Detected	Detected	Not Detected	Multiple infections are possible but rare, retest to confirm result [a]
Influenza A/H1, A/H1pdm09, and A/H3 Detected	Detected	Detected	Detected	Detected	Multiple infections are possible but rare, retest to confirm result [a]
Influenza A (no subtype detected)	Detected	Not Detected	Not Detected	Not Detected	Retest (see the section on Influenza A, no subtype detected below)

Target Assay / BioCode RPP Result	FluA	A/H1	A/H1pdm09	A/H3	Action
Influenza A Indeterminate	Not Detected	Detected or Not Detected	Detected or Not Detected	Detected	Retest [b]
	Not Detected	Detected	Detected or Not Detected	Detected or Not Detected	
	Not Detected	Detected or Not Detected	Detected	Detected or Not Detected	

[a] Repeated multiple positive should be further confirmed by other FDA-cleared influenza subtyping assays.
[b] If the re-test result confirms the original result, it is recommended that the sample be further investigated using a different FDA-cleared influenza A subtyping assay and/or sending the residual sample to local public health laboratory for further testing.

Influenza A (no subtype detected):

If the FluA assay is positive, but none of the hemagglutinin (HA) subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain or a seasonal Influenza A/H3 or A/H1pdm09 strain with critical sequence mismatches to the primers and/or probes of the BioCode RPP influenza A HA subtyping assays. In both cases, the sample in question should be retested. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the BioCode RPP should be verified by testing with appropriate external control materials (known positive samples for Influenza A/H1, Influenza A/H3 and Influenza A/H1pdm09), and a negative control should also be run to test for PCR-product contamination. If the BioCode RPP accurately identifies the external positive and negative controls, contact the appropriate public health authorities for confirmatory testing.

BioCode RPP Reports

The analyzed BioCode MDx-3000 results are displayed in two report formats: Run Report for the entire run including multiple specimens, and Sample Report for individual specimens. Both reports can be exported as a PDF or CSV file. Each report includes fully analyzed and interpreted results for specimens and/or controls but is formatted differently.

The Run Report displays analyzed results in a tabular format for all wells (specimens/controls) in a run from a specific kit lot. If more than one lot is run together, separate Run Reports will be generated by the software for each lot. Possible results by target assay are: Detected, Not detected, Invalid, Indeterminant (for Influenza A only), or N/A (if not ordered).

The Sample Report displays results for a single well (specimen/control). In addition to results for each target assay, the Sample Reports include a results summary section which allows positive results to be reviewed at a glance. The Sample Report results summary will also indicate well validity based on BMB counts, background MFI, and external and internal controls. Sample reports also include any sample-specific comments entered during setup.

Both report headers provide traceability information for: run name, run start and finish time, user ID, software version, instrument ID, kit name, and reagent lots with expiration dates. The report headers also include sections for Run Status and Controls Status. The Run Status section will specify if the run is Incomplete, Valid or Invalid (based on the External Negative Control results for the specific run/kit lot). The Controls Status section indicates the results for the External Negative Controls (Valid or Invalid) and External Positive Controls (Valid, Invalid, or N/A if not assayed). The Run Status and Controls Status sections should be reviewed prior to review of target assay results. In addition, the software will also mask results in the detailed tabular sections based on plate and well validity requirements.

Completed reports can be reviewed electronically. Reviewer comments can be added to the report footer for traceability under the review section. In addition, MFI reports are available with information only for administrator level users.

B Principle of Operation:

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

Nucleic Acid Extraction

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

Overview of a BioCode MDx-3000 Run:

Reverse Transcription and Multiplex PCR

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

PCR and Product Transfer

For the PCR amplification, the robotic head dispenses BMB- Probe mix into the designated reaction wells of the capture plate using disposable pipette tips. After PCR amplification is completed, the robotic head pierces the foil seal with disposable pipette tips and transfers PCR products into corresponding wells of the capture plate.

Target Capture

Amplified PCR products labeled with biotin are captured at a defined temperature by target-specific probes that are covalently coupled to designated Barcoded Magnetic Beads (BMBs).

During this step, BMBs are kept in suspension by gentle agitation. Differentiation of captured targets is achieved by assigning a unique barcode pattern of BMBs for each pathogen and the Internal Control.

Signal Generation

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

Optical Detection

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

Interpretation of Results

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

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☒	☐
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name:
   BioCode MDx-3000 Instrument

2. Specimen Identification:
   Specimen identity is provided by barcode magnetic beads.

3. Specimen Sampling and Handling:
   After extraction with the NucliSENS easyMag system or the MagNA Pure 96 system and manually loading samples into a 96-well formatted plate, the BioCode MDx 3000 processes all RT-PCR, target capture, signal generation, and optical detection steps automatically.

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

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

V Substantial Equivalence Information:

A Predicate Device Name(s):
FilmArray Respiratory Panel 2 (RP2)

B Predicate 510(k) Number(s):
K170604

C Comparison with Predicate(s):

Similarities or Differences		
Element	**BioCode Respiratory Pathogen Panel (RPP) (K192485)**	**FilmArray Respiratory Panel 2 (RP2) (K170604)**
Specimen Types	NPS (in VTM or UTM)	Same
Pathogens Detected	Adenovirus, Coronavirus (229E, HKU1, NL63, and OC43), Human Metapneumovirus A/B, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 Pandemic, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Rhinovirus/Enterovirus, Respiratory Syncytial Virus A/B, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*	Same, except that the four coronaviruses, 229E, HKU1, NL63, and OC43, are detected and reported separately, and the pathogens detected also include *Bordetella parapertussis*
Analyte	RNA/DNA	Same
Technological Principles	Multiplex nucleic acid	Same
Instrumentation	BioCode MDx-3000 Instrument, bioMérieux NucliSENS easyMAG system or Roche MagNA Pure 96 system	FilmArray 2.0 or FilmArray Torch
Time to Result	About 5.0 hours	About 45 minutes
Test Interpretation	Automated test interpretation and report generation.	Same

Controls	An RNA Internal Control is added to each sample during extraction. External Positive and Negative Controls are externally sourced.	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis. External Positive and Negative Controls are externally sourced.
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 PCR 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 melting curve analyses of the PCR reactions.
CLIA Complexity	High	Moderate

VI Standards/Guidance Documents Referenced:

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   A reproducibility study was conducted at three testing sites using a combination of NucliSENS easyMAG and MagNA Pure 96 automated systems for nucleic acids extraction (two sites used NucliSENS easyMAG and one site used MagNA Pure 96 for nucleic acids extraction). The study incorporated potential sources of variation introduced by site (three testing sites), day (five different days), and operator (two operators per site). One lot of reagents was assayed at three sites by two operators on one BioCode MDx-3000 instrument per site for five days. A total of 10 BioCode RPP runs were performed per site.

   A total of six contrived NPS samples containing known quantities of various BioCode RPP analytes (Table 5 below) were prepared in a simulated NPS in UTM sample matrix. A NPS sample negative for all BioCode RPP analytes was also prepared using the simulated NPS in UTM sample matrix. The six contrived positive samples consisted of combinations of 12

representative pathogens, at 1.5x LoD (Low) and 3x LoD (Medium), spiked into a simulated NPS in UTM sample matrix.

Table 5: Reproducibility Study Panel – Sample Composition

Medium (3x LoD)	Medium (3x LoD)	Low (1.5x LoD)	Low (1.5x LoD)	Sample Name
Rhinovirus	Parainfluenza Virus 2	Human Metapneumovirus	*Bordetella pertussis*	RP1
Human Metapneumovirus	*Bordetella pertussis*	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 NPS in UTM matrix				RP7

Information regarding the specific strains and isolates of the 12 representative pathogens and concentrations that were used in building the reproducibility study panel samples are provided in Table 6 below.

Table 6: Reproducibility Study Panel - Strains/Isolates and Concentrations

Pathogen	Strain/Serotype	Source/ ID	Limit of Detection (LoD)	Low Concentration (1.5xLoD)	Medium Concentration (3xLoD)
Coronavirus NL63	NL63	Zeptometrix 0810228CF	0.04 $TCID_{50}$/mL	0.06 $TCID_{50}$/mL	0.12 $TCID_{50}$/mL
Parainfluenza virus 2	Greer/Ohio/1955	ATCC VR-92	5.4 $TCID_{50}$/mL	8.1 $TCID_{50}$/mL	16.2 $TCID_{50}$/mL
Adenovirus C	Species C	ATCC VR-846	18.0 $TCID_{50}$/mL	27.0 $TCID_{50}$/mL	54.0 $TCID_{50}$/mL
Influenza A H3N2	A/Wisconsin/67/05	Zeptometrix 0810252CF	4.0 $TCID_{50}$/mL	6.0 $TCID_{50}$/mL	12.0 $TCID_{50}$/mL
Human Rhinovirus	Type 1A	Zeptometrix 0810012CF	1.2 $TCID_{50}$/mL	1.8 $TCID_{50}$/mL	3.6 $TCID_{50}$/mL
Mycoplasma pneumoniae	M129	Zeptometrix 0801579	15.0 CCU/mL	22.5 CCU/mL	45.0 CCU/mL
Chlamydia pneumoniae	AR39	ATCC VR-53592	33.3 CFU/mL	50.0 CFU/mL	100.0 CFU/mL
Influenza B	Florida/4/2006 (Yamagata)	Zeptometrix 0810255CF	0.01 $TCID_{50}$/mL	0.02 $TCID_{50}$/mL	0.03 $TCID_{50}$/mL
Parainfluenza virus 3	N/A	Zeptometrix 0810060CF	15.0 $TCID_{50}$/mL	22.5 $TCID_{50}$/mL	45.0 $TCID_{50}$/mL
Human Metapneumovirus	Type 16, Type A1 IA10-2003	Zeptometrix 0810161CF	15.0 $TCID_{50}$/mL	22.5 $TCID_{50}$/mL	45.0 $TCID_{50}$/mL
Respiratory Syncytial Virus	Type A	Zeptometrix 0810040ACF	0.33 $TCID_{50}$/mL	0.45 $TCID_{50}$/mL	0.9 $TCID_{50}$/mL
Bordetella pertussis	A639	Zeptometrix 0801459	45.0 CFU/mL	67.5 CFU/mL	135.0 CFU/mL

Once prepared, each sample of the reproducibility study panel was tested with the BioCode RPP to confirm that it contained the intended analytes at the intended concentration and then divided into single-use aliquots and stored frozen (≤ -70˚C) until the day of testing.

After being distributed to the sites, each sample was extracted in triplicates using either the NucliSENS easyMAG (Site 1 and Site 3) or the MagNA Pure 96 (Site 2) and assayed using the BioCode RPP. In total, 90 data points per sample were obtained, with 60 data points generated by the NucliSENS easyMAG system (Site 1 and Site 3) and 30 data points generated by the MagNA Pure 96 system.

A summary of the reproducibility study results, percent (%) agreement with the expected Detected or Not Detected result, for each analyte (by site and extraction system) is provided in Table 7 below.

Table 7: Reproducibility of BioCode RPP

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS easyMAG			MagNA Pure 96		All Sites (95% CI)
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	
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** 99.3%	**299/300** 99.7%	**150/150** 100%	**150/150** 100%	**449/450** **99.8%** (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%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS easyMAG			MagNA Pure 96		All Sites (95% CI)
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	
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] 96.7%	**30/30** 100%	**59/60** 98.3%	**30/30** 100%	**30/30** 100%	**89/90** **98.9%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**149/150** [b] 99.3%	**299/300** 99.7%	**150/150** 100%	**150/150** 100%	**449/450** **99.8%** (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** 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** 96.7%	**30/30** 100%	**59/60** 98.3%	**30/30** 100%	**30/30** 100%	**89/90** **98.9%** (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%)

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS easyMAG			MagNA Pure 96		All Sites (95% CI)
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	
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%)
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%)
Respiratory Syncytial Virus	**3× LoD**	**Detected**	**29/30** 100%	**30/30** 100%	**59/60** 98.3%	**30/30** 100%	**30/30** 100%	**89/90** **98.9%** (94.0%-99.8%)
	1.5× LoD	**Detected**	**29/30** 100%	**30/30** 100%	**59/60** 98.3%	**30/30** 100%	**30/30** 100%	**89/90** **98.9%** (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%)
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** 99.3%	**299/300** 99.7%	**150/150** 100%	**150/150** 100%	**449/450** **99.8%** (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%)

[a] There was an indeterminate Flu A result for Influenza A/H3 low positive sample.

[b] There was an indeterminate Flu A result for Influenza A/H3 negative sample.

2. Linearity:
   Not applicable, qualitative assay

3. Assay Reportable Range:
   Not applicable, qualitative assay

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

Assay Controls

External Negative Controls
External Negative Controls are user provided. They can be RNase-free water, transport media, or well characterized negative specimens. The External Negative Controls should go through all processing steps (extractions, amplification, and detection). At least one External Negative Control is required for each plate/kit lot. The BioCode MDx-3000 software will suppress results for all samples if the External Negative Control is not valid.

External Positive Controls
External Positive Controls are user provided. They can be well characterized clinical samples or cultured organisms. External Positive Controls can be single analytes or pooled multianalyte specimens. External Positive Controls should go through all processing steps (extractions, amplification, and detection). It is recommended that at least one External Positive Control be included for each plate/kit lot on a rotating schedule. Wells identified as External Positive Controls will be trended by the BioCode MDx-3000 software and the report will indicate a valid or invalid result on the report header. However, the software will not suppress results based on External Positive Control results. If an External Positive Control does not perform as expected, the user should review all samples in that plate to determine if results can be reported.

Internal Control
An RNA Internal Control (RNA IC: bacteriophage MS2) is provided in the reagent kit and is added to each sample during extraction. The Internal Control monitors the efficiency of the extraction, reverse transcription, amplification, and detection stages of the assay. Positive target assay results may be reported in the absence of RNA IC detection. However, the BioCode MDx-3000 software will suppress target assay negative results for any wells with invalid RNA IC results.

External controls are not provided with the BioCode RPP. However, six frozen (-70°C) external control mixes obtained from ZeptoMetrix as NATtrol controls for different pathogens (see Table 8 below) were provided to the clinical study sites for testing during the prospective clinical study and the clinical study testing retrospective and contrived specimens. Operators were required to follow the BioCode RPP instructions for use during the testing.

Table 8: External Control Mixes Utilized in the Clinical Evaluations

External Control Mixes	Expected Calls
PC-A	Influenza B, Respiratory Syncytial Virus, Parainfluenza Virus 1, and Rhinovirus/Enterovirus
PC-B	Coronavirus HKU1, Parainfluenza Virus 2, Human Metapneumovirus, and *Bordetella pertussis*
PC-C	Influenza A/H1, Coronavirus NL63, and Adenovirus
PC-D	Influenza A/H1pdm09, Parainfluenza Virus 4, Coronavirus OC43, and *Chlamydia pneumoniae*
PC-E	Influenza A/H3, Parainfluenza Virus 3, Coronavirus 229E, and *Mycoplasma pneumoniae*.
NC	Negative for all analytes

Performance of testing the external controls during the clinical studies are summarized in Table 9 below.

Table 9: External Controls Testing Summary

	Agreement with Expected Results					
	PC-A	**PC-B**	**PC-C**	**PC-D**	**PC-E**	**NC**
NucliSENS easyMAG	9/10 [a]	8/9 [a]	6/6	11/11	8/8	50/53 [b]
MagNA Pure 96	4/4	5/5	4/4	3/3	3/3	20/20
Total	13/14	13/14	10/10	14/14	11/11	70/73

[a] PC and NC switched during loading due to operator's error.

[b] Of the three failed NCs, one was due to RNA IC failure, one due to operator's error (NC and PC switched during loading), and one due to detection of an unexpected target.

Specimen Stability Study

Nasopharyngeal Swab (NPS) should be collected according to standard technique and immediately placed in 1 – 3 mL of VTM or UTM. Samples should be tested as soon as possible. An analytical study was performed to assess the specimen stability limitations for the optimal performance of the BioCode RPP on the BioCode MDx-3000. Nine representative pathogens from the BioCode RPP were spiked into prescreened negative natural NPS in UTM specimens at 3x LoD (see Table 10 below) and assayed with three extraction replicates on the easyMAG at each timepoint, except for baseline. A total of seven extraction replicates on the easyMAG were assayed at timepoint 0. Stability of extracted nucleic acids was also assessed in this study.

Table 10: Contrived Sample in Prescreened Negative Natural NPS in UTM Utilized in the Specimen Stability Study

Sample Name	Pathogen Name and Concentration
RP A	Adenovirus (ADV at 3xLoD), *Mycoplasma pneumoniae* (MPN at 3xLoD), and Influenza A/H3 (Flu A/H3 at 3xLoD)
RP B	Respiratory Syncytial Virus (RSV at 3xLoD), Influenza A/H1pdm09 (Flu A/H1pdm09 at 3xLoD), and Human Metapneumovirus (hMPV at 3xLoD)
RP C	Parainfluenza Virus 3 (PIV 3 at 3xLoD), Coronavirus NL63 (CoV-NL63 at 3xLoD), and Influenza B (Flu B at 3xLoD)

Specimen stability study summary results are presented in Table 11 below.

Table 11: Specimen Stability Study Summary Results

Sample Type	Temperature	Time (hours or Days)	Agreement with Expected Results								
			Sample RP A			Sample RP B			Sample RP C		
			ADV	MPN	Flu A/H3	RSV	Flu A/H1 pdm09	hMPV	PIV 3	CoV-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
Contrived 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 Acids	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

The specimen stability testing data supports the following specimen stability claims in the product package insert:

NPS collected in VTM or UTM may be stored at room temperature for 8 hours, in a 2-8°C refrigerator for 7 days, or at <-60°C for up to 90 days.

Extracted nucleic acids may be stored in a 2-8°C refrigerator for up to 12 hours, or at ≤-60°C for up to 90 days.

Fresh vs. Frozen

The results of the Specimen Stability Study (see Table 11 above) also demonstrated that preservation of samples (by freezing at -80°C) does not affect the accuracy of test results compared to freshly prepared samples. Therefore, it is appropriate to utilize frozen archived prospective and retrospective clinical samples in the evaluation of BioCode RPP to supplement the prospective (fresh) clinical study data, and to use frozen simulated samples in analytical studies for this submission.

Single-Spiked vs. Multi-Spiked Specimen Study

An analytical study was conducted 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. LoD was determined for single spiked samples in simulated NPS for four representative pathogens on both the easyMAG and MagNA pure extraction systems. For initial range finding, four replicates of each concentration were extracted on the easyMAG and MagNA pure systems and tested in singlet with the BioCode RPP on the BioCode MDx-3000 system to estimate LoD. The LoD was further confirmed by extracting 20 replicates of each sample and testing each in singlet for a total of 20 replicates at or near the presumptive LoD. The single spiked LoDs were then challenged by pooling all

four pathogens and testing at 1x LoD with 20 extraction replicates on both extraction systems. When 1x LoD for each pathogen in the mixed pool does not meet the 95% detection goal, the pooled pathogen samples were retested at 3x LoD with 20 extraction replicates. Results within 3x LoD were considered to be equivalent in this study.

Summary results of this study are presented in Table 12 below.

Table 12: Single-Spiked vs. Multi-Spiked Study Summary Results

Pathogen	Target Assay	Single-Spiked or Multi-Spiked	NucliSENS easyMAG		MagNA Pure 96	
			Concentration	Detection (n/20)	Concentration	Detection (n/20)
Influenza A/H3N2	Flu A	Single-Spiked	1.3 $TCID_{50}$/mL	20/20	1.3 $TCID_{50}$/mL	20/20
	Flu A/H3			20/20		20/20
	Flu A	Multi-Spiked	4.0 $TCID_{50}$/mL	20/20		20/20
	Flu A/H3			20/20		20/20
Coronavirus NL63	NL 63	Single-Spiked	0.013 $TCID_{50}$/mL	20/20	0.040 $TCID_{50}$/mL	19/20
		Multi-Spiked	0.040 $TCID_{50}$/mL	20/20		20/20
Mycoplasma pneumoniae	MPN	Single-Spiked	5.0 CCU/mL	19/20	15.0 CCU/mL	20/20
		Multi-Spiked	15.0 CCU/mL	20/20		20/20
Adenovirus C	ADV1	Single-Spiked	6.0 $TCID_{50}$/mL	20/20	18.0 $TCID_{50}$/mL	20/20
		Multi-Spiked		20/20		20/20

The acceptance criteria of this study were met since all pathogens tested were within 3xLoD for single-spiked and multi-spiked samples. The study results seem to demonstrate that the performance of the BioCode RPP on the BioCode MDx-3000 with mixed analyte samples at or near the Limit of Detection (LoD) is similar to single spiked samples. Using multiple analyte spiked samples during analytical validation testing appears to be acceptable.

Simulated vs. Natural NPS in UTM Specimen Study

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.

Summary results of this analytical study are presented in Table 13 below.

Table 13: Simulated vs. Natural NPS in UTM Specimen Study Summary Results

Pools	Pathogen	Concentration	Detected (N of 4)			
			NucliSENS easyMAG		MagNA Pure 96	
			Natural NPS in UTM	sNPS in UTM	Natural NPS in UTM	sNPS in UTM
A	Adenovirus E Serotype 4	0.6 $TCID_{50}$/mL (15xLoD)	4/4	4/4	4/4	4/4
	Chlamydia pneumoniae	75.0 CFU/mL (2.3 - 4.5xLoD)	4/4	4/4	4/4	4/4
	Influenza A/H3N2	6.0 $TCID_{50}$/mL (1.5 -4.6xLoD)	4/4	4/4	4/4	4/4
B	Influenza A/H1N1	67.5 $TCID_{50}$/mL (4.5 – 13.5xLoD)	4/4	4/4	4/4	4/4
	Respiratory Syncytial Virus A	1.5 $TCID_{50}$/mL (4.5xLoD)	4/4	4/4	4/4	4/4
	Human Metapneumovirus	67.5 $TCID_{50}$/mL (4.5xLoD)	4/4	4/4	4/4	4/4
C	Influenza B	0.06 $TCID_{50}$/mL (6.0xLoD)	4/4	4/4	4/4	4/4
	Coronavirus OC43	0.06 $TCID_{50}$/mL (1.5 – 6.0xLoD)	4/4	4/4	4/4	4/4
	Parainfluenza Virus 4	13.5 $TCID_{50}$/mL (1.5xLoD)	4/4	4/4	4/4	4/4
Negative Control		N/A	4/4	4/4	4/4	4/4

The study results show that the performance testing contrived samples in this simulated NPS (sNPS) in UTM matrix compared to testing contrived samples in natural NPS in UTM matrix using the BioCode RPP, with both the NucliSENS easyMAG and the MagNA Pure 96 extraction systems, are similar. Therefore, it is acceptable to utilize the simulated NPS in UTM sample matrix in conducting the analytical studies in support of this submission.

5. Detection Limit:

Limit of detection (LoD) estimation and confirmation studies were carried out with contrived samples in simulated NPS in UTM (Remel M4 transport medium) matrix designed to resemble a natural clinical NPS in UTM specimen. An equivalence study was performed which demonstrated that the simulated matrix was similar to the natural clinical NPS matrix and did not impact BioCode RPP test performance. Refer to the "Simulated vs. Natural NPS in UTM Specimen Study" section.

Representative isolates of respiratory viruses and bacteria were selected to make contrived samples in order to obtain positive results for every target assay on the panel. In some cases, testing of more than one isolate/strain per analyte was performed to assess LoD for clinically important species or variants, specifically when more than one assay was needed to detect the expected diversity of an analyte (e.g. Adenovirus).

An estimated LoD concentration for each analyte was first determined by testing serial dilutions of contrived samples. Four extraction replicates per dilution on each extraction system, NucliSENS easyMAG and MagNA Pure 96, were tested using the BioCode RPP. The LoD was confirmed by extracting 20 replicates on each extraction system and testing each for a total of 20 replicates at or near the estimated LoD using the BioCode RPP. LoD for each isolate was defined as the lowest concentration with ≥95% detection of 20 replicates (at least 19 out of 20 replicates).

A multi-spiked approach was employed in both the LoD estimation and the LoD confirmation studies where samples spiked with two pathogens at various concentrations were tested in the LoD studies. An equivalence study was performed which demonstrated that the performance of the multi-spiked samples was similar to that of the single-spiked

samples and employing a multi-spiking approach did not impact BioCode RPP test performance. Refer to the "Single-Spiked vs. Multi-Spiked Specimen Study" section.

Summary results of the LoD confirmation study are presented in Table 14 below.

Table 14: Limit of Detection Confirmation by Extraction System

Pathogen	Strain/Isolate	Source	NucliSENS easyMAG		MagNA Pure 96	
			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/H1pdm09	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	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	Geer/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
	Species C Serotype 2	ATCC VR-846	6.0 $TCID_{50}$/mL	20/20	18.0 $TCID_{50}$/mL	20/20
	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
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 with Applied BioCode validated SYBR assay using an IVT RNA standard.

6. Analytical Reactivity:

An analytical study was performed to assess analytical reactivity/inclusivity of the BioCode RPP. Different strains, serotypes and genotypes were selected that represent temporal, geographic, and genetic diversity for each pathogen. A panel of titered stocks for relevant organisms/viruses diluted in simulated NPS in UTM matrix were tested starting at 3x LoD. Organisms/viruses not detected at 3x LoD were tested at higher concentrations. Since the confirmed LoD result for B/Hong Kong/S/1972 (Victoria lineage) was higher than expected at 48.6 $TCID_{50}$/mL, which could be related to difference in titration from vendor sources, testing of the influenza B Victoria strains in this study was performed starting at 0.03xLoD or 0.3xLoD. Strains of unknown lineages of influenza B were tested at 3x LoD for the B/Florida/4/2006 strain of the Yamagata lineage.

Each sample was extracted in triplicate on the NucliSENS easyMAG and tested with the BioCode RPP on the BioCode MDx-3000 according to the instructions for use.

Note: Although this analytical reactivity testing was performed in part to demonstrate that the BioCode RPP can detect various species and strains of a pathogen with similar analytical sensitivity, it is difficult to make comparative sensitivity evaluations between different organisms and different strains that have been quantified in $TCID_{50}$/mL or EID_{50}/mL. This quantification method measures the infectivity and cytotoxicity or lethality of a strain in tissue culture or in chick embryo, and is therefore subject to many influences (e.g., strain-to-strain differences in infectivity, viability of the original material, culturing conditions, etc.). Quantification by infectivity assay will not be equivalent between strains or organisms. Also, since the measurand for the BioCode RPP is nucleic acids, LoD concentrations established in $TCID_{50}$/mL or EID_{50}/mL can vary dramatically among different strains but may not actually reflect significant differences in analytical reactivity of detection as measured by target nucleic acids concentration.

Analytical reactivity testing results are summarized in Table 15 to Table 25 below.

Table 15: Adenovirus Isolates Tested and Detected by BioCode RPP

Species [a]	Serotype	Isolate ID/Source	Strain/Location/Year	xLoD Detected	Result
A	31	Zeptometrix 0810073CF	Unknown	3x	Adenovirus Detected
	12	ATCC VR-863	Huie/Massachusetts	3x	
	18	ATCC VR-19	Washington DC/1954	3x	
B	3	ATCC VR-3	GB/Maryland/1953	3x	
	14	Zeptometrix 0810108CF	Unknown	3x	
	16	ATCC VR-17	CH.79/Saudi Arabia/1955	3x	
	35	ATCC VR-718	Holden	3x	
C	1	Zeptometrix 0810050CF	Unknown	3x	
	5	Zeptometrix 0810020CF	Unknown	3x	
	6	ATCC VR-6	Tonsil 99/Washington DC	3x	
D	8	Zeptometrix 0810069CF	Unknown	3x	
	17	ATCC VR-1836	CH.22/Saudi Arabia/1955	3x	
	20	Zeptometrix 0810115CF	Unknown	3x	
	26	Zeptometrix 0810117CF	Unknown	3x	
	37	Zeptometrix 0810119CF	Unknown	3x	
F	40	Zeptometrix 0810084CF	Unknown	3x	
	41	ATCC VR-930	Tak/73-3544/Netherlands/1973	3x	

[a] In addition to the Adenovirus species E serotype 4 strain tested in the LoD study, *in silico* analysis of available sequences predicts that the BioCode RPP will also react with other Adenovirus species E serotypes.

Table 16: Coronavirus Isolates/Specimens Tested and Detected by BioCode RPP

Coronavirus Type	Isolate ID/Source	Location/Year	xLoD Detected	Result
229E	ATCC VR-740	Unknown	3x	Coronavirus Detected
NL63	BEI NR-470 [a]	Amsterdam/2003	3x	Coronavirus Detected
OC43	ATCC VR-1558	Unknown	3x	Coronavirus Detected
HKU1	Clinical Sample 5016	Unknown	3x	Coronavirus Detected
	Clinical Sample 5036	Unknown	3x	
	Clinical Sample 5037	Unknown	10x	

[a] Organism obtained through BEI Resources, NIAID, NIH: Human Coronavirus NL63, NR-470.

Table 17: Human Metapneumovirus Isolates Tested and Detected by BioCode RPP

Genotype	Serotype	Isolate ID/Source	Location/Year	xLoD Detected	Result
A1	9	Zeptometrix 0810160CF	Iowa3/2002	3x	Human Metapneumovirus Detected
A2	27	Zeptometrix 0810164CF	Iowa27/2004	3x	
B1	3	Zeptometrix 0810156CF	Peru2/2002	3x	
	5	Zeptometrix 0810158CF	Peru3/2003	3x	
B2	4	Zeptometrix 0810157CF	Peru1/2002	3x	
	8	Zeptometrix 0810159CF	Peru6/2003	3x	
	18	Zeptometrix 0810162CF	Iowa18/2003	3x	
	Unknown	BEI NR-22232 [a]	TN/91-316	3x	

[a] Virus obtained through BEI Resources, NIAID, NIH: Human Metapneumovirus, TN/91-316, NR-22232.

Table 18: Human Rhinovirus and Enterovirus Isolates Tested and Detected by BioCode RPP

Species	Serotype/Strain/Isolate	Source	xLoD Detected	Result
Human Rhinovirus				
A	Serotype 7 [68-CV11]	ATCC VR-1601	3x	Human Rhinovirus/ Enterovirus Detected
	Serotype 16 [1A]	Zeptometrix 0810285CF	3x	
	Serotype 16 [11757/Washington DC/1960]	ATCC VR-283	3x	
	Serotype 34 [173-3]	ATCC VR-1365	3x	
	Serotype 57 [ch47]	ATCC VR-1600	3x	
	Serotype 77 [130-63]	ATCC VR-1187	3x	
	Serotype 80	Zeptometrix 0810288CF	3x	
	Serotype 85 [50-525-CV54]	ATCC VR-1195	3x	
	Serotype 95 [SF-998]	ATCC VR-1301	3x	
B	Serotype 3 [FEB]	ATCC VR-483	3x	
	Serotype 14	Zeptometrix 0810284CF	3x	
	Serotype 42	Zeptometrix 0810286CF	3x	
	Serotype 70	Zeptometrix 0810287CF	3x	
Enterovirus				
A	Enterovirus 71	Zeptometrix 0810236CF	3x	Human Rhinovirus/ Enterovirus Detected

Table 19: Influenza A Isolates Tested and Detected by BioCode RPP

Influenza A Type [a]	Strain	Source	xLoD Detected	Result
Influenza A H1N1	Solomon Island/03/06	Zeptometrix 0810036CFN	3x	Influenza A/H1 Detected
	Singapore/63/04	Zeptometrix 0810246CF	3x	
	PR/8/34	Zeptometrix 0810245CF	3x	
	A/Brisbane/59/2007	Zeptometrix 0810244CF	3x	
	A/Taiwan/42/06	Zeptometrix 0810247CF	3x	
	A/New jersey/8/76	ATCC VR-897	500x [b]	
	A/Denver/1/1957	VIRAPUR	3x	
	A/FM/1/47	ATCC VR-97	3x	
	A/Weiss/43	ATCC VR-96	1000x [c]	
	A/Beijing/262/95 [d]	BEI NR-12277	30x [e]	
	A/Mal/302/54	ATCC VR-98	3x	
Influenza A H1N2	Recombinant; Kilbourne F64, A/NWS/1934 (HA) x A/Rockefeller Institute/ 5/1957 (NA) [f]	BEI NR-3682	5x [g]	
Influenza A H1N1 pdm09	NY/01/09	Zeptometrix 0810248CF	3x	Influenza A/H1 pdm09 Detected
	NY/02/09	Zeptometrix 0810109CFN	3x	
	NY/03/09	Zeptometrix 0810249CF	3x	
	A/Houston/3H/2009 (H1N1) pdm09 [h]	BEI NR-20340	3x	
	Influenza A H1N1pdm09 (Canada/6294/09)	Zeptometrix 0810109CFJ	3x	
	Influenza A H1N1pdm09 (Mexico/4108/09)	Zeptometrix 0810166CF	3x	
	California/04/09, cell isolate [i]	BEI NR-13658	100x [j]	
	A/Christ Church/16/2010	CDC 2010839805	1000x [k]	
	A/Brisbane/02/2018	CDC 3000683658	100x [l]	
Influenza A H3N2	A/Wisconsin/15/2009 [m]	BEI NR-42007	3x	Influenza A/H3 Detected
	A/Texas/50/12	Zeptometrix 0810238CF	3x	

	A/Brisbane/10/2007	Zeptometrix 0810138CF	3x	
	A/Port Chalmers/1/73	ATCC VR-810	50x [n]	
	A/Victoria/3/1975	VIRAPUR	3x	
	A/Victoria/361/2011 [o]	BEI NR-44022	3x	
	A/Victoria/3/75	ATCC VR-822	3x	
	A/Uruguay/716/07 [p]	BEI NR-42003	10x	
	A/HK/H090-756-V1(0)/2009 [q]	BEI NR-44344	3x	
	A/Hong Kong/8/68	Zeptometrix 0810250CF	3x	
	A/Switzerland/9715293/13	VIRAPUR	3x	
	A/Aichi/2/68	ATCC VR-547	3x	
	MRC-2	ATCC VR-777	3x	
	A/Perth/16/2009	CDC 2009719818	10x	
	A/Kansas/14/2017	CDC 3026015402	2000x [r]	

[a] *In silico* analysis supports 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] Detected as Flu A (no subtype) at 3x LoD. Detected as dual positive A/H1 and A/H1pdm09 at 500x LoD.

[c] 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] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Beijing/262/95 (H1N1), NR-12277.

[e] Detected as Flu A (no subtype) at 10xLoD. *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.

[f] 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

[g] Detected as Flu A (no subtype) at 3x LoD.

[h] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Houston/3H/2009 (H1N1) pdm09, NR-20340

[i] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/California/04/09, cell isolate (H1N1) pdm09, NR-13658

[j] *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.

[k] 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.

[l] 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.

[m] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Wisconsin/15/2009 (H3N2), NR-42007

[n] *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.
[o] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Victoria/361/2011 (H3N2), NR-44022
[p] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Uruguay/716/07 (H3N2), NR-42003
[q] Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/HK/H090-756-V1(0)/2009 (H3N2), NR-44344
[r] 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. 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. Therefore, 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.

Table 20: Influenza B Isolates Tested and Detected by BioCode RPP

Lineage	Strain	Source	xLoD Detected	Result
Unknown	B/Lee/1940	Zeptometrix 0810257CF	3x	Influenza B Detected
	B/Taiwan/2/1962	ATCC VR-295	500x [a]	
	B/Allen/45	ATCC VR-102	5x	
	B/Brigit/Russia/1969	ATCC VR-786	3x	
Victoria [b]	B/Malaysia/2506/2004 [c]	BEI NR-9723	0.03x	
	B/Malaysia/2506/2004	Zeptometrix 0810258CF	0.03x	
	B/Ohio/01/2005 [d]	BEI NR-41801	0.3x	
	B/Brisbane/33/2008 [e]	BEI NR-42006	0.3x	
	B/Nevada/03/2011 [f]	BEI NR-44023	0.03x	
	B/Michigan/09/2011	CDC 2012700901	0.3x	
	B/Colorado/06/2017	CDC 3025629447	0.3x	
Yamagata	B/Texas/06/2011 [g]	BEI NR-44024	5000x [h]	
	B/Sydney/507/2006 [i]	BEI NR-36526	800x [j]	
	B/Wisconsin/1/10	Zeptometrix 0810241CF	3x	
	B/Massachusetts/2/12	Zeptometrix 0810239CF	3x	
	B/Christchurch/33/2004 [k]	BEI NR-36536	3x	
	B/New Hampshire/01/2016 [l]	CDC 3000415764	1000x [l]	
	B/Phuket/3073/2013[m]	CDC 2014768616	500x [m]	

[a] *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.
[b] For Victoria strains testing started at 0.03x LoD rather than 3x LoD.
[c] Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Malaysia/2506/2004, NR-9723
[d] Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Ohio/01/2005, NR-41801
[e] Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Brisbane/33/2008, NR-42006
[f] Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Nevada/03/2011, NR-44023
[g] Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Texas/06/2011, NR-44024
[h] *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
[j] *In silico* analysis does not predict reduced analytical reactivity. Titering inconsistency from the vendor rather than reduced reactivity due to assay design is suggested.
[k] Virus obtained through BEI Resources, NIAID, NIH: Influenza B, B/Christchurch/33/2004, NR-36536
[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] 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.

Table 21: Parainfluenza Virus Isolates Tested and Detected by BioCode RPP

Type	Subtype	Strain	Source	xLoD Detected	Result
1		FRA/27344044/2007 [a]	BEI NR-48681	3x	Parainfluenza Virus 1 Detected
		FRA/29221106/2009 [b]	BEI NR-48680	3x	
		Unknown	Zeptometrix 0810014CF	3x	
2		Greer [c]	BEI NR-3229	3x	Parainfluenza Virus 2 Detected
		Unknown	Zeptometrix 0810015CF	3x	
3		NIH 47885, Wash/47885/57 [d]	BEI NR-3233	3x	Parainfluenza Virus 3 Detected
		C-243/Washington DC/1957	ATCC VR-93	3x	
4	a	M-25/1958 [e]	BEI NR-3237	3x	Parainfluenza Virus 4 Detected
	b	CH-19503/Washington DC/1962	ATCC VR-1377	3x	
		Unknown	Zeptometrix 0810060BCF	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 22: Respiratory Syncytial Virus Isolates Tested and Detected by BioCode RPP

Type	Strain	Source	xLoD Detected	Result
A	TN/1998/3-2 [a]	BEI NR-28529	3x	Respiratory Syncytial Virus Detected
	TN/2000/3-4 [b]	BEI NR-28530	10x	
	TN/98/12-21 [c]	BEI NR-28528	3x	
	Long/Maryland/1956	ATCC VR-26	10x	
B	9320/Massachusetts/1977	ATCC VR-955	3x	
	B1[d]	BEI NR-4052	10x	
	WV/14617/1985	ATCC VR-1400	3x	
	18537/Washington DC/1962	ATCC VR-1580	3x	
	CH-93 (18)-18	Zeptometrix 0810040CF	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 23: *Bordetella pertussis* Isolates Tested and Detected by BioCode RPP

Isolate	Source	xLoD Detected	Result
F	ATCC 8467	3x	*Bordetella pertussis* Detected
5[17921]	ATCC 9340	3x	
10-536	ATCC 10380	3x	
CNCTC Hp 12/63,623	ATCC 51445	3x	
Tohama 1	ATCC BAA-589	3x	
MN2531	ATCC BAA-1335	3x	

Table 24: *Chlamydia pneumoniae* Isolates Tested and Detected by BioCode RPP

Isolate	Source	xLoD Detected	Result
CM-1/Georgia	ATCC VR-1360	3x	*Chlamydia pneumoniae* Detected
CWL-029	ATCC VR-1310	3x	

Table 25: *Mycoplasma pneumoniae* Isolates Tested and Detected by BioCode RPP

Isolate	Source	xLoD Detected	Result
M129-B7	ATCC 29342	3x	*Mycoplasma pneumoniae* Detected
PI 1428	ATCC 29085	3x	

Mac	ATCC 15492	3x	
UTMB-10P	ATCC 49894	3x	

Analytical reactivity was demonstrated with a wide variety of isolates/strains.

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.

7. Analytical Specificity/Cross-Reactivity:

Analytical specificity (cross-reactivity) of the BioCode RPP was evaluated by challenging the system with contrived samples in simulated NPS in UTM matrix containing a high concentration of the potentially cross-reacting organism. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity (e.g., does the Coronavirus OC43 assay cross-react with Coronavirus HKU1, etc.), while off-panel organisms (those not intended to be detected by the panel) were tested to assess the potential for non-specific amplification of respiratory flora or other respiratory pathogens that may be present in an NPS specimen.

Each organism was tested at 10^6 CFU/mL for bacteria or fungi and 10^5 $TCID_{50}$/mL for viruses or higher when possible at Applied BioCode, Inc. Organism or virus stocks were spiked in simulated NPS in UTM matrix at desired concentrations at the time of extraction. Each sample was extracted in triplicate on the NucliSENS easyMAG and assayed in singlet with the BioCode 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 five additional extraction replicates. If detected after repeat with five additional replicates, serial dilutions were performed to determine the lower limit.

On-Panel Organisms/Viruses Testing

A group of 26 on-panel analytes were tested at high concentration to assess the potential for intra-panel cross-reactivity. The organisms or viruses tested are shown in Table 26 below.

Table 26: On-panel Organisms/Viruses Tested by the BioCode RPP for Evaluation of Analytical Specificity

Organism/Virus	Source	Titer Tested	Cross-Reactivity Observed (Y/N)
Influenza A H1N1/New Caledonia/20/99	Zeptometrix 0810036CF	1.15×10^5 TCID $/mL_{50}$	N
Influenza A H1N1/NWS/33	ATCC VR-219	7.40×10^5 TCID $/mL_{50}$	N
Influenza A H1N1 pdm09/California/07/09	Zeptometrix 0810165CF	1.31×10^5 TCID $/mL_{50}$	N
Influenza A H3N2/Wisconsin/67/05a	Zeptometrix 0810252CF	1.08×10^5 TCID $/mL_{50}$	N
Influenza A H3N2/Alice	ATCC VR-776	1.43×10^6 TCID $/mL_{50}$	N
Influenza B/Florida/4/2006 (Yamagata)	Zeptometrix 0810255CF	1.08×10^5 TCID $/mL_{50}$	N
Influenza B/Hong Kong/S/1972 (Victoria)	ATCC VR-823	8.57×10^5 TCID $/mL_{50}$	N
Respiratory Syncytial Virus (Type A)	Zeptometrix 0810040ACF	4.57×10^5 TCID $/mL_{50}$	N
Human Metapneumovirus 16 (Type A1)	Zeptometrix 0810161CF	8.51×10^5 TCID $/mL_{50}$	N
Parainfluenza Virus 1	ATCC VR-94	1.60×10^5 TCID $/mL_{50}$	N
Parainfluenza Virus 2	ATCC VR-92	1.35×10^5 TCID $/mL_{50}$	N
Parainfluenza Virus 3	Zeptometrix 0810016CF	3.39×10^5 TCID $/mL_{50}$	N
Parainfluenza Virus 4a	Zeptometrix 0810060CF	1.13×10^5 TCID $/mL_{50}$	N
Adenovirus Species B Serotype 7A	Zeptometrix 0810021CF	5.83×10^5 TCID $/mL_{50}$	N
Adenovirus Species C Serotype 2	ATCC AV-846	2.81×10^5 TCID $/mL_{50}$	N
Adenovirus Species E Serotype 4	Zeptometrix 0810070CF	1.08×10^5 TCID $/mL_{50}$	N
Coronavirus 229E	Zeptometrix 0810229CF	1.09×10^5 TCID $/mL_{50}$	N
Coronavirus HKU1	Clinical Sample [a]	1.92×10^5 Copies/mL	N
Coronavirus NL63	Zeptometrix 0810228CF	1.08×10^5 TCID $/mL_{50}$	N
Coronavirus OC43	Zeptometrix 0810024CF	1.08×10^5 TCID $/mL_{50}$	N

Human Rhinovirus Type A1	Zeptometrix 0810012CF	1.05 x 10^5 TCID /mL_{50}	N
Enterovirus D68	Zeptometrix 0810300CF	1.08 x 10^5 TCID /mL_{50}	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 sample quantified with Applied BioCode validated SYBR assay using an IVT RNA standard.

Off-Panel Organisms/Viruses Testing

In silico analysis of assay specificity was supplemented with wet testing of 52 off-panel bacteria, viruses, and fungi at high concentrations (typically 10^6 CFU/mL for bacteria or fungi and 10^5 $TCID_{50}$/mL for viruses or higher when possible). The organisms and viruses tested are shown in Table 27 below.

Table 27: Off-panel Organisms and Viruses Tested by the BioCode RPP for Evaluation of Analytical Specificity

Organism/Virus	Source	Titer Tested	Cross-Reactivity Observed (Y/N)
Acinetobacter baumannii	Zeptometrix 801597	9.67 x 10^6 CFU/mL	N
Aspergillus flavus	Zeptometrix 801598	1.72 x 10^6 CFU/mL	N
Bordetella bronchiseptica	Zeptometrix 801649	6.68 x 10^7 CFU/mL	N
Bordetella holmesii	Zeptometrix 801464	3.83 x 10^0 CFU/mL	Y [a]
Bordetella parapertussis	Zeptometrix 8011461	1.00 x 10^6 CFU/mL	N
Burkholderia cepacia	Zeptometrix 801584	4.13 x 10^7 CFU/mL	N
Candida albicans	Zeptometrix 801504	1.96 x 10^6 CFU/mL	N
Candida glabrata	Zeptometrix 801535	1.73 x 10^7 CFU/mL	N
Corynebacterium diphtheriae	Zeptometrix 801882	4.57 x 10^6 CFU/mL	N
Haemophilus influenzae	Zeptometrix 801679	2.40 x 10^6 CFU/mL	N
Klebsiella pneumoniae	Zeptometrix 801506	5.10 x 10^7 CFU/mL	N
Lactobacillus plantarum	Zeptometrix 801507	1.80 x 10^7 CFU/mL	N
Legionella longbeachae	Zeptometrix 8101577	1.93 x 10^7 CFU/mL	N
Legionella micdadei	Zeptometrix 801576	2.70 x 10^7 CFU/mL	N
Legionella pneumophila	Zeptometrix 801645	3.17 x 10^7 CFU/mL	N
Moraxella catarrhalis	Zeptometrix 801509	1.13 x 10^6 CFU/mL	N
Mycobacterium tuberculosis	Zeptometrix 801660	7.23 x 10^6 CFU/mL	N
Mycoplasma genitalium	ATCC 33530	1.00 x 10^3 CFU/mL	N
Mycoplasma hominis	ATCC 23114	2.7 x 10^4 CFU/mL	N
Neisseria elongata	Zeptometrix 801510	1.74 x 10^7 CFU/mL	N
Neisseria gonorrhoeae	Zeptometrix 801482	1.26 x 10^7 CFU/mL	N
Neisseria meningitidis	Zeptometrix 801511	2.55 x 10^6 CFU/mL	N
Neisseria sicca	Zeptometrix 801754	1.02 x 10^6 CFU/mL	N

Organism/Virus	Source	Titer Tested	Cross-Reactivity Observed (Y/N)
Proteus vulgaris	Zeptometrix 801898	4.13 x 10^7 CFU/mL	N
Pseudomonas aeruginosa	ATCC 39324	2.11 x 10^6 CFU/mL	N
Serratia marcescens	Zeptometrix 801723	2.06 x 10^7 CFU/mL	N
Staphylococcus haemolyticus	Zeptometrix 801591	8.50 x 10^6 CFU/mL	N
Streptococcus agalactiae	Zeptometrix 801545	3.73 x 10^6 CFU/mL	N
Streptococcus dysgalactiae	Zeptometrix 801516	1.23 x 10^6 CFU/mL	N
Streptococcus intermedius	Zeptometrix 801895	5.07 x 10^6 CFU/mL	N
Streptococcus mitis	Zeptometrix 801695	5.73 x 10^6 CFU/mL	N
Streptococcus pneumoniae	Zeptometrix 801439	4.17 x 10^6 CFU/mL	N
Ureaplasma urealyticum	ATCC 27618	1.00 x 10^7 CFU/mL	N
Coxsackievirus A10	Zeptometrix 0810106CF	1.05 x 10^3 $TCID_{50}$/mL	Y [b]
Coxsackievirus A21	Zeptometrix 0810235CF	$\leq$ 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	$\leq$ 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	$\leq$ 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
SARS-CoV (formaldehyde and UV-inactivated, vaccine 1%)	BEI NR-3883	1:100 Dilution	N
MERS-CoV (EMC/2012, heat-inactivated)	BEI NR-50171	2.00 x 10^5 $TCID_{50}$/mL	N
MERS-CoV gRNA	BEI NR-45843	1.01 x 10^7 Copies/mL	N

[a] *Bordetella holmesii* was detected by the *Bordetella pertussis* (BP) assay in 2 of 3 replicates down to 3.83 x 10^0 CFU/mL.
[b] The Coxsackieviruses assayed were detected by the Human Rhinovirus/Enterovirus (HRV) assay in at least 1 of 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 of the 3 replicates down to the concentrations indicated.

Analytical Specificity Evaluation Conclusion

Analytical specificity testing has demonstrated that the majority of the BioCode RPP assays are highly specific for the organisms/viruses they are designed to detect. Cross-reactivity that was observed during the testing was almost exclusively associated with near-neighbor species that carry the same genes or highly similar

sequences as the targeted pathogen. Overall, the likelihood, risk, and impact of the BioCode RPP non-specific interactions are predicted to be minor.

The BioCode RPP includes assays to distinguish classical human Influenza A H1 and the A H1 2009 pandemic variant derived from swine. However, due to sequence similarity, some reactivity of the A/H1pdm09 assay may be observed with historical and/or novel A/H1N1 strains of swine origin. This was demonstrated by testing a swine origin influenza A A/New Jersey/8/1976 in the analytical reactivity study which was detected and reported as dual positive for Influenza A/H1 and Influenza A/H1pdm09 when tested at a concentration of 7.5×10^3 $CEID_{50}$/mL (approximately 500xLoD).

Coxsackievirus and Echovirus may be detected as Human Rhinovirus/Enterovirus even at low concentrations. Due to the genetic similarity between Human Rhinovirus and Coxsackievirus and Echovirus, this expected.

Other non-pertussis *Bordetella* species that harbor the repetitive element IS*481* sequence or other related sequence variants, *Bordetella holmesii* and *Bordetella bronchiseptica* in particular, may be detected as *Bordetella pertussis* by the BioCode RPP.

All identified BioCode RPP cross-reactivity cases are indicated in the product Instructions for Use, as a precaution to minimize misinterpretation of results. A summary is provided in Table 28 below.

Table 28: Predicted and Observed Cross-Reactivity of the BioCode RPP

Cross-reactive Organism	BioCode RPP Result	Description
Non-pertussis *Bordetella* species that harbor IS*481* sequence, *Bordetella holmesii* and *Bordetella bronchiseptica* in particular	*Bordetella pertussis*	The *Bordetella pertussis* assay may amplify IS*481* sequences in non-pertussis *Bordetella* species, such as *B. bronchiseptica* and *B. holmesii*.
Influenza A H1N1 (swine origin)	Influenza A/H1pdm09	The Influenza A/H1pdm09 assay may react with H1 hemagglutinin gene sequences from viruses of swine origin. BioCode RPP results may be either Influenza A/H1 or Influenza A/H1pdm09, depending on the strain and concentration in the sample.

8. Assay Cut-Off:

Preliminary MFI cutoff values were determined by analyzing the results of 500 archived frozen NPS samples during verification based on two main criteria, signal to noise ratio and the relative levels of sensitivity and specificity based on the Receiver Operating Characteristic (ROC) analysis. The cutoff MFIs selected should produce a signal to noise ratio of ≥5 for 95% of test wells and a Sensitivity ≥95% and Specificity ≥95% based on the ROC analysis with an invalid rate (RNA IC) of ≤2%.

After completion of the analytical validation and clinical sample testing, the data was reviewed to determine if any of the cutoff values established initially could be improved. This review prompted the change of the ADV2 assay cutoff from 500 to 10000 MFI.

Table 29 below summarizes the MFI cutoff values for the BioCode RPP.

Table 29: Summary of Final MFI Cutoff Values for Each BMB-Probe Assay

BMB-Probe Assay	Cutoff (≥)	BMB-Probe Assay	Cutoff (≥)	BMB-Probe Assay	Cutoff (≥)
Blank	<3000	hMPV1	500	CoV-229E	300
Flu A	500	hMPV2	500	CoV-HKU1	400
Flu A/H1	1000	PIV1	500	CoV-NL63	500
Flu A/H1pdm09	500	PIV2	400	CoV-OC43	500
Flu A/H3	1000	PIV3	500	HRV	300
Flu B1	500	PIV4	500	BP	500
Flu B2	500	ADV1	500	MPN	500
RSV	500	ADV2	10000	CPN	1000
				RNA IC	8000

9. Interfering Substances:

An analytical study was performed to assess potential inhibitory effects of substances and microorganisms that may be commonly found in nasopharyngeal specimens. Each member of the interfering substance and microorganism panel was added to simulated NPS (sNPS) matrix spiked with representative members of the BioCode RPP (see Table 30 below) at 3x LoD and a negative matrix comprised of only sNPS. Each sample was tested with and without potentially interfering substances or microbes. Each sample was prepared and extracted in triplicate on both NucliSENS easyMAG and MagNA Pure 96 extraction system and tested with the BioCode RPP on the BioCode MDx-3000 system. Substances that produce interference at the original test concentration were further tested at lower concentrations.

Table 30: Contrived Samples (3x LoD in sNPS)

Sample Name	Organism/Virus	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

In total, 30 different endogenous and exogenous substances, potentially competing microorganisms, specimen collection materials (swabs and media), and disinfecting agents were evaluated in this study (see Table 31 below).

Table 31: Summary Results of the Interference Study

Substance Interferent	Brand/Source	Concentration Tested	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
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
Microbial Interferent	**Source**	**Concentration Tested**	**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

[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.

In addition, nasal influenza vaccine Flu Mist was also evaluated in this study. It was tested without extraction in triplicates. Testing results are presented in Table 32 below.

Table 32: Nasal Influenza Vaccine (FluMist) Tested with the BioCode RPP

FluMist 2010-2011 (V/V%)	Influenza A			Influenza B
	A/H1	A/H1pdm09	A/H3	
10%	-	+	+	+
1%	-	+	+	+
0.1%	-	+	+	+
0.01%	-	+	+	+
0.001%	-	+	+	+
0.0001%	-	+	+ [a]	+
0.00001%	-	-	-	+ [a]
0.000001%	-	-	-	-

[a] 2/3 replicates detected.

This study showed that none of the substances interfered with BioCode RPP at the concentrations tested. All substances and microorganisms tested appeared to be compatible with BioCode RPP assay 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 the NucliSENS easyMAG system. 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.

10. Carry-Over Contamination:
A formal carry-over study in support of this regulatory submission for the BioCode RPP was not performed, since carry-over studies with high positive samples followed by negative samples have been performed for the FDA-cleared BioCode GPP using both the NucliSENS easyMAG (K180041) and the MagNA Pure 96 systems (K190585). No carry-over has been observed.

11. Competitive Inhibition:
An analytical study was performed to evaluate the potential for inhibition in samples with mixed infections. Simulated NPS (sNPS) in UTM matrix was spiked with one target at high concentration ($\geq 10^6$ CFU/mL or CCU/mL for bacteria and $\geq 10^5$ $TCID_{50}$/mL for viruses) and two targets at low concentrations ($\leq$ 3x LoD).

Common respiratory tract co-infections were determined by reviewing results of previous Respiratory Pathogen Panel clinical trials from published 510k summaries, publications/posters and internal clinical sample testing. Each sample was extracted in triplicate using the NucliSENS easyMAG and each extraction tested in singlet with the BioCode RPP on the BioCode MDx-3000 system.

Summary results of this study are presented in Table 33 below. No inhibition was observed at the concentrations tested in this study.

Table 33: Competitive Inhibition Study Results

Panel Designation	Viral/Bacteria Strain	Level	Titer Tested	Result (n of 3 Detected)
Competitive Inhibition Sample 1	Adenovirus C Serotype 2	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Respiratory syncytial virus Type A	Low	0.99 $TCID_{50}$/mL	3/3
	Influenza A H3N2 A/Wisconsin/67/05	Low	12 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 2	Respiratory syncytial virus Type A	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza A H3N2 A/Wisconsin/67/05	Low	12 $TCID_{50}$/mL	3/3
	Adenovirus C Serotype 2	Low	18 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 3	Influenza A H3N2 A/Wisconsin/67/05	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Adenovirus C Serotype 2	Low	18 $TCID_{50}$/mL	3/3
	Respiratory syncytial virus Type A	Low	0.99 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 4	Coronavirus OC43	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Human Metapneumovirus	Low	45 $TCID_{50}$/mL	3/3
	Bordetella pertussis	Low	45 CFU/mL	3/3
Competitive Inhibition Sample 5	Human Metapneumovirus	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Bordetella pertussis	Low	45 CFU/mL	3/3
	Coronavirus OC43	Low	0.12 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 6	*Bordetella pertussis*	High	$1x10^6$ CFU/mL	3/3
	Coronavirus OC43	Low	0.12 $TCID_{50}$/mL	3/3
	Human Metapneumovirus	Low	45 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 7	Influenza A H1N1 pdm California/07/09	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Parainfluenza Virus 3	Low	45 $TCID_{50}$/mL	3/3
	Human Rhinovirus type A	Low	3.6 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 8	Parainfluenza Virus 3	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Human Rhinovirus type A	Low	3.6 $TCID_{50}$/mL	3/3
	Influenza A H1N1 pdm California/07/09	Low	1.2 $TCID_{50}$/mL	3/3

Competitive Inhibition Sample 9	Human Rhinovirus type A	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza A H1N1 pdm California/07/09	Low	1.2 $TCID_{50}$/mL	3/3
	Parainfluenza Virus 3	Low	45 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 10	*Mycoplasma pneumoniae*	High	$1x10^6$ CCU/mL	3/3
	Coronavirus NL63	Low	1.2 $TCID_{50}$/mL	3/3
	Influenza B/Florida/4/2006	Low	0.04 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 11	Coronavirus NL63	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza B/Florida/4/2006	Low	0.04 $TCID_{50}$/mL	3/3
	Mycoplasma pneumoniae	Low	45 CCU/mL	3/3
Competitive Inhibition Sample 12	Influenza B/Florida/4/2006	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Mycoplasma pneumoniae	Low	45 CCU/mL	3/3
	Coronavirus NL63	Low	1.2 $TCID_{50}$/mL	3/3

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable. Refer to the Clinical Studies Section of this document.

2. Matrix Comparison:
   Not applicable

C Clinical Studies:

1. Prospective Clinical Study

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 (Category I specimens, 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 (Category II specimens, 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 Category I and 1248 Category II 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.

Table 34: Specimen Enrollment Sites and Extraction Methods for the BioCode RPP Prospective Clinical Evaluation

Site	Study Site Location	Category I Specimen Collected	Category II Specimen Collected	Total Number of Prospective Specimens (Category I and II)	Extraction NucliSENS easy MAG	Extraction MagNA Pure 96
1	New York City, NY	250	280	530	530	0
2	Memphis, TN	182	237	419	0	419
3	Tampa, FL	366	234	600	600	0
4	Los Angeles, CA	300	250	550	550	0
5	Aurora, CO	303	247	550	0	550
Total		1401	1248	2649	1680	969

Table 35 below provides a summary of demographic information for the 2649 specimens included in the prospective clinical study.

Table 35: Demographic Summary for the Prospective BioCode RPP Clinical Evaluation

		Overall	Site 1	Site 2	Site 3	Site 4	Site 5
Sex	Male	1346 (50.8%)	271	195	266	320	294
	Female	1303 (49.2%)	259	224	334	230	256
Age	≤ 5 years	1004 (37.9%)	18	161	91	360	374
	6 - 21 years	609 (23.0%)	32	172	45	187	173
	22 - 59 years	531 (20.0%)	190	65	271	3	2
	60+ years	505 (19.1%)	290	21	193	0	1
Total		**2649**	**530**	**419**	**600**	**550**	**550**

Prospective Clinical Study System and Specimen Validity Rate

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%).

Prospective Clinical Study Performance

Performance of the BioCode RPP was evaluated by comparing the BioCode RPP test results with those of an FDA-cleared molecular multiplexed respiratory pathogen panel. Positive percent agreement (PPA) was calculated as TP/(TP + FN) (TP = true positive or positive by both the comparator method and BioCode RPP; FN = false negative or negative by BioCode RPP only). Negative percent agreement was calculated as TN/(TN + FP) (TN = true negative or negative by both the comparator method and BioCode RPP; FP = false positive or positive by BioCode RPP only). The score binomial two-sided 95% confidence interval was also calculated. 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 method 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 Category I and one Category II specimens, obtained a final "invalid" result from the BioCode RPP, and were excluded from the performance analyses for all analytes. In addition, three specimens, one Category I and two Category II specimens, obtained a final influenza A "indeterminate" result by the BioCode RPP, and two specimens, one Category I and one Category II specimens obtained an influenza A "equivocal" result from the comparator method. 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 Category II specimens obtained a valid influenza A result by the comparator method 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 BioCode RPP prospective performance data as positive percent and negative percent agreements against the comparator method (all sites combined) are presented by pathogen in Table 36 below:

Table 36: BioCode RPP Prospective Clinical Performance Summary

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
Adenovirus [a]	Fresh	31/40	77.5	62.5-87.7	1340/1360	98.5	97.7-99.0
	Frozen	37/38	97.4	86.5-99.5	1188/1209	98.3	97.4-98.9
	Overall	***68/78***	***87.2***	***78.0-92.9***	***2528/2569***	***98.4***	***97.8-98.8***
Coronavirus [b]	Fresh	35/50	70.0	56.2-80.9	1338/1350	99.1	98.5-99.5
	Frozen	76/83	91.6	83.6-95.9	1154/1164	99.1	98.4-99.5
	Overall	***111/133***	***83.5***	***76.2-88.8***	***2492/2514***	***99.1***	***98.7-99.4***
hMPV [c]	Fresh	89/93	95.7	89.5-98.3	1299/1307	99.4	98.8-99.7
	Frozen	46/49	93.9	83.5-97.9	1189/1198	99.2	98.6-99.6
	Overall	***135/142***	***95.1***	***90.2-97.6***	***2488/2505***	***99.3***	***98.9-99.6***
HRV/EV [d]	Fresh	221/261	84.7	79.8-88.5	1119/1139	98.2	97.3-98.9
	Frozen	162/213	76.1	69.9-81.3	1020/1034	98.6	97.7-99.2
	Overall	***383/474***	***80.8***	***77.0-84.1***	***2139/2173***	***98.4***	***97.8-98.9***
FluA [e]	Fresh	115/120	95.8	90.6-98.2	1265/1278	99.0	98.3-99.4
	Frozen	98/101	97.0	91.6-99.0	1131/1143	99.0	98.2-99.4
	Overall	***213/221***	***96.4***	***93.0-98.2***	***2396/2421***	***99.0***	***98.5-99.3***
FluA/H1	Fresh	0/0	0	N/A	1398/1398	100	99.7-100
	Frozen	0/0	0	N/A	1242/1242	100	99.7-100
	Overall	***0/0***	***0***	***N/A***	***2640/2640***	***100***	***99.9-100***
FluA/H1pdm09 [f]	Fresh	29/30	96.7	83.3-99.4	1365/1368	99.8	99.4-99.9
	Frozen	23/23	100	85.7-100	1213/1219	99.5	98.9-99.8
	Overall	***52/53***	***98.1***	***90.1-99.7***	***2578/2587***	***99.7***	***99.3-99.8***
FluA/H3 [g]	Fresh	82/88	93.2	85.9-96.8	1306/1310	99.7	99.2-99.9
	Frozen	65/69	94.2	86.0-97.7	1168/1173	99.6	99.0-99.8
	Overall	***147/157***	***93.6***	***88.7-96.5***	***2474/2483***	***99.6***	***99.3-99.8***
FluB [h]	Fresh	7/7	100	64.6-100	1388/1393	99.6	99.2-99.8
	Frozen	44/47	93.6	82.8-97.8	1191/1200	99.2	98.6-99.6
	Overall	***51/54***	***94.4***	***84.9-98.1***	***2579/2593***	***99.5***	***99.1-99.7***
PIV1 [i]	Fresh	4/4	100	51.0-100	1396/1396	100	99.7-100
	Frozen	11/13	84.6	57.8-95.7	1234/1234	100	99.7-100
	Overall	***15/17***	***88.2***	***65.7-96.7***	***2630/2630***	***100***	***99.9-100***
PIV2 [j]	Fresh	2/3	66.7	20.8-93.9	1396/1397	99.9	99.6-100
	Frozen	8/9	88.9	56.5-98.0	1236/1238	99.8	99.4-100
	Overall	***10/12***	***83.3***	***55.2-95.3***	***2632/2635***	***99.9***	***99.7-100***
PIV3 [k]	Fresh	77/79	97.5	91.2-99.3	1312/1321	99.3	98.7-99.6
	Frozen	41/43	95.3	84.5-98.7	1196/1204	99.3	98.7-99.7
	Overall	***118/122***	***96.7***	***91.9-98.7***	***2508/2525***	***99.3***	***98.9-99.6***

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
PIV4 [l]	Fresh	1/1	100	20.7-100	1399/1399	100	99.7-100
	Frozen	15/17	88.2	65.7-96.7	1228/1230	99.8	99.4-100
	Overall	***16/18***	***88.9***	***67.2-96.9***	***2627/2629***	***99.9***	***99.7-100***
RSV [m]	Fresh	91/93	97.8	92.5-99.4	1293/1307	98.9	98.2-99.4
	Frozen	109/111	98.2	93.7-99.5	1129/1136	99.4	98.7-99.7
	Overall	***200/204***	***98.0***	***95.1-99.2***	***2422/2443***	***99.1***	***98.7-99.4***
Bacteria							
***B. pertussis* [n]**	Fresh	1/1	100	20.7-100	1387/1399	99.1	98.5-99.5
	Frozen	1/1	100	20.7-100	1239/1246	99.4	98.8-99.7
	Overall	***2/2***	***100***	***34.2-100***	***2626/2645***	***99.3***	***98.9-99.5***
***C. pneumoniae* [o]**	Fresh	2/2	100	34.2-100	1397/1398	99.9	99.6-100
	Frozen	2/2	100	34.2-100	1245/1245	100	99.7-100
	Overall	***4/4***	***100***	***51.0-100***	***2642/2643***	***100***	***99.8-100***
***M. pneumoniae* [p]**	Fresh	8/8	100	67.6-100	1381/1392	99.2	98.6-99.6
	Frozen	10/10	100	72.2-100	1228/1237	99.3	98.6-99.6
	Overall	***18/18***	***100***	***82.4-100***	***2609/2629***	***99.2***	***98.8-99.5***

[a] Adenovirus: PCR/bi-directional sequencing and alternative NAAT were performed on all FNs. PCR/bi-directional sequencing was also performed on all FPs. Of 10 FNs, none was detected by PCR/bi-directional sequencing or alternative NAAT. Of 41 FPs, 37 were not detected by PCR/bi-directional sequencing; 4 were indeterminate by PCR/bi-directional sequencing.

[b] Coronavirus: PCR/bi-directional sequencing was performed on all FNs and FPs. Alternative NAAT was performed on 14/19 FNs only, but not on FPs. Of 22 FNs, 5 were detected and 17 were not detected by PCR/bi-directional sequencing. 2 were detected and 12 were not detected by alternative NAAT. The 22 FPs were not detected by PCR/bi-directional sequencing.

[c] Human Metapneumovirus: PCR/bi-directional sequencing was performed on all FNs and FPs. Of 7 FNs, 3 were detected and 4 were not detected by PCR/bi-directional sequencing. Of 17 FPs, 7 were detected and 10 were not detected by PCR/bi-directional sequencing.

[d] Human Rhinovirus/Enterovirus: PCR/bi-directional sequencing was performed on all FNs and FPs. Alternative NAAT was also performed on 36/91 FNs, but not on FPs. Of 91 FNs, 63 were not detected by PCR/bi-directional sequencing; 18 were detected as HRV C, 1 as HRV A, 2 as Enterovirus D68, 5 as Coxsackieviruses, and 2 as indeterminate HRV C, by PCR/bi-directional sequencing. 25 of 36 FNs that were also tested by alternative NAAT generated a positive result for HRV/EV. Of 34 FPs, 26 were not detected by PCR/bi-directional sequencing; 3 were detected as HRV A, 1 as HRV B, 3 as HRV C, and 1 as Enterovirus D68, by PCR/ bi-directional sequencing.

[e] Influenza A: PCR/bi-directional sequencing was performed on 7/8 FNs and 24/25FPs. Of 7 FNs that were tested by PCR/bi-directional sequencing, all 7 were not detected by PCR/bi-directional sequencing. Of 24 FPs that were tested by PCR/bi-directional sequencing, 19 were not detected by, 4 were detected as Flu A H3N2, and 1 was detected as Flu A H1N1pdm09.

[f] Influenza A/H1pdm09: PCR/bi-directional sequencing was performed on all FPs. Of 9 FPs, 4 were detected by PCR/bi-directional sequencing; 5 were not detected by PCR/bi-directional sequencing.

[g] Influenza A/H3: PCR/bi-directional sequencing was performed on 8/10 FNs and 8/9 FPs. Of 8 FNs that were tested by PCR/bi-directional sequencing, 7 were not detected, and 1 was detected. Of 8 FPs that were tested by PCR/bi-directional sequencing, 3 were detected, 1 was indeterminate, and 4 were not detected.

[h] Influenza B: PCR/bi-directional sequencing was performed on all FNs and 13/14 FPs. Of 3 FNs, none was detected by PCR/bi-directional sequencing. Of 13 FPs that were tested by PCR/bi-directional sequencing, 1 was detected and 12 were not detected.

[i] Parainfluenza Virus 1: The 2 FNs were not detected by PCR/bi-directional sequencing.

[j] Parainfluenza Virus 2: PCR/bi-directional sequencing was performed on all FPs and FNs. Of 2 FNs, 1 was detected and 1 was not detected by PCR/bi-directional sequencing. All 3 FPs were detected by PCR/bi-directional sequencing.

[k] Parainfluenza Virus 3: PCR/bi-directional sequencing was performed on all FPs and FNs. Of 4 FNs, 2 were detected by PCR/bi-directional sequencing; 1 was indeterminate by PCR/bi-directional sequencing; and 1 was not

detected by PCR/bi-directional sequencing. Of 17 FPs, 8 were detected by PCR/bi-directional sequencing; 1 was indeterminate by PCR/bi-directional sequencing; and 8 were not detected by PCR/bi-directional sequencing.
[l] Parainfluenza Virus 4: PCR/bi-directional sequencing was performed on all FPs and FNs. All 2 FNs and 2 FPs were detected by PCR/bi-directional sequencing.
[m] Respiratory Syncytial Virus: PCR/bi-directional sequencing was performed on all FPs and FNs. The 4 FNs were not detected by PCR/bi-directional sequencing. Of 21 FPs, 18 were not detected by PCR/bi-directional sequencing; 2 were detected by PCR/bi-directional sequencing; 1 was indeterminate by PCR/bi-directional sequencing.
[n] *Bordetella pertussis:* Of 19 FPs, 4 were detected by PCR/bi-directional sequencing; 3 were indeterminate by PCR/bi-directional sequencing; and 12 were not detected by PCR/bi-directional sequencing.
[o] *Chlamydia pneumoniae:* The 1 FP was detected by PCR/bi-directional sequencing.
[p] *Mycoplasma pneumoniae:* PCR/bi-directional sequencing was performed on 18/20 FPs. Of 18 FPs that were tested by PCR/bi-directional sequencing, 7 were detected, 1 was invalid, and 10 were not detected.

The performance of the BioCode RPP overall testing of Category I specimens was similar to that of testing Category II specimens. In addition, the performance of the BioCode RPP testing prospective specimens was also analyzed stratified by study sites and by extraction methods. No significant performance differences between the two extraction methods, NucliSENS easyMAG and MagNA Pure 96, or among the five study sites, were observed.

Prospective Clinical Study Influenza A and Subtyping Analysis

A total of 15 specimens generated an initial "Influenza A Indeterminate" result. 14 of the 15 samples had sufficient residual volume and were retested per the instructions for use. Upon retest, 12 of the 14 specimens obtained a final unequivocal Influenza A and subtyping result and two of the 14 specimens remained as "Influenza A Indeterminate". The percentage of samples that reported a final "Influenza A Indeterminate" result after retest was 0.1% (3/2647).

A total of 29 specimens generated an initial "Influenza A (no subtype detected)" result. 15 of the 29 samples had sufficient residual volume and were retested per the instructions for use. Upon retest, six of the 15 samples remained to be "Influenza A (no subtype detected)", one of the 15 samples generated an Influenza A/H3 result, and eight of the 15 samples generated an Influenza A negative result. Out of a total of 238 specimens that obtained a final Influenza A positive result after retest, 20 specimens were reported as "Influenza A (no subtype detected)" (8.4%, 20/238).

Prospective Clinical Study Mixed Infection Analysis

BioCode RPP reported a total of 193 specimens with distinctive multiple pathogen detections (7.3% of all specimens, 193/2647; and 9.4% of all positive specimens, 193/2050). The majority of multiple detections, 87.0% (168/193) contained two pathogens, while 12.4% (24/193) contained three pathogens, 0.5% (1/193) contained more than three pathogens.

The three pathogens that were most prevalent in multiple detections were HRV/EV (101/193, 52.3%), RSV (59/193, 30.1%), and adenovirus (51/193, 26.4%) (see Table 37 below).

Table 37: Prevalence of Analytes in Multiple Detections as Determined by the BioCode RPP

Analyte	Prevalence in Multiple Detections (N=193)	
Viruses		
Adenovirus	51	26.4%
Coronavirus	47	24.4%
hMPV	35	18.1%
HRV/EV	101	52.3%
Flu A	37	19.2%
Flu B	8	4.1%
PIV1	2	1.0%
PIV2	4	2.1%
PIV3	39	20.2%
PIV4	8	4.1%
RSV	59	30.1%
Bacteria		
B. pertussis	14	7.3%
C. pneumoniae	1	0.5%
M. pneumoniae	7	3.6%

A summary of specimens with co-infections as detected by the BioCode RPP during the prospective clinical study is presented in Table 38 below.

Table 38: Co-infections Detected by the BioCode RPP in the Prospective Clinical Trial

Pathogens Detected Simultaneously	Detected Co-infections	Discrepant Co-infections [a]
2	168	83
3	24	9
4	0	0
5	1	1
Total Co-infections	193	93

[a] A discrepant sample of co-infection was defined as a sample that contains at least one pathogen that was detected by BioCode RPP but not detected by the comparator method.

A summary of the most prevalent co-infections as detected by the BioCode RPP (≥ 5 instances) during the prospective clinical study is presented in Table 39 below

Table 39: Most Prevalent Co-infections Detected by the BioCode RPP (≥ 5 Instances) in the Prospective Clinical Trial

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
Adenovirus + Parainfluenza Virus 3	5
Coronavirus + Human Metapneumovirus	5
Coronavirus + Influenza A	5

2. Retrospective Clinical Study

Several 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 the five testing sites participated in the prospective clinical study for testing.

A summary of the demographic information of the tested retrospective samples is provided in Table 40 below.

Table 40: Available Demographic Summary for All Retrospective Specimens Included in the Retrospective Study

Sex	Female (%)	69 (41.8%)
	Male (%)	96 (58.2%)
Age	≤ 5 years	77 (46.7%)
	6 - 21 years	58 (35.2%)
	22 - 59 years	15 (9.1%)
	60+ years	15 (9.1%)
Total Specimens		**165**

Retrospective Clinical Study Performance

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 Table 41 below.

Table 41: BioCode RPP Retrospective Clinical Study Performance Summary

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Adenovirus [a]	7/7	100	64.6-100	155/158	98.1	94.6-99.4
Coronavirus [b]	52/59	88.1	77.5-94.1	99/106	93.4	87.0-96.8
hMPV	4/4	100	51.0-100	161/161	100	97.7-100
HRV/EV [c]	16/23	69.6	49.1-84.4	141/142	99.3	96.1-99.9
Influenza A	0/0	0	N/A	165/165	100	97.7-100
Influenza A/H1	0/0	0	N/A	165/165	100	97.7-100
Influenza A/H1pdm09	0/0	0	N/A	165/165	100	97.7-100
Influenza A/H3	0/0	0	N/A	165/165	100	97.7-100
Influenza B [d]	2/3	66.7	20.8-93.9	162/162	100	97.7-100
Parainfluenza Virus 1 [e]	12/13	92.3	66.7-98.6	152/152	100	97.5-100
Parainfluenza Virus 2 [f]	19/20	95.0	76.4-99.1	144/145	99.3	96.2-99.9
Parainfluenza Virus 3	1/1	100	20.7-100	164/164	100	97.7-100
Parainfluenza Virus 4 [g]	14/15	93.3	70.2-98.8	150/150	100	97.5-100
RSV [h]	11/12	91.7	64.6-98.5	152/153	99.3	96.4-99.9
Bacteria						
Bordetella pertussis [i]	10/10	100	72.2-100	144/155	92.9	87.7-96.0
Chlamydia pneumoniae	10/10	100	72.2-100	155/155	100	97.6-100
Mycoplasma pneumoniae [j]	7/7	100	64.6-100	153/158	96.8	92.8-98.6

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

[b] Coronavirus: PCR/bi-directional sequencing was performed on all FNs and FPs. Of 7 FNs, 4 were detected and 3 were not detected by PCR/bi-directional sequencing. Of 7 FPs, none was detected by PCR/bi-directional sequencing.
[c] Human Rhinovirus/Enterovirus: PCR/bi-directional sequencing was performed on all FNs and FPs. Of 7 FNs and 1 FP, none was detected by PCR/bi-directional sequencing.
[d] Influenza B: 1 FN was not detected by PCR/bi-directional sequencing.
[e] Parainfluenza Virus 1: 1 FN was not detected by PCR/bi-directional sequencing
[f] Parainfluenza Virus 2: PCR/bi-directional sequencing was performed on FN and FP samples. 1 FN was not detected by PCR/bi-directional sequencing. 1 FP was not detected by PCR/bi-directional sequencing.
[g] Parainfluenza Virus 4: 1 FN was not detected by PCR/bi-directional sequencing.
[h] Respiratory Syncytial Virus: PCR/bi-directional sequencing was performed on FN and FP samples. 1 FN and 1 FP were not detected by PCR/bi-directional sequencing.
[i] *Bordetella pertussis*: PCR/bi-directional sequencing was performed on all FPs. Of 11 FPs, 7 were detected by PCR/bi-directional sequencing, and 4 were not detected by PCR/bi-directional sequencing.
[j] *Mycoplasma pneumoniae*: PCR/bi-directional sequencing was performed on all FPs. Of 5 FPs, 3 were detected by PCR/bi-directional sequencing and 2 was not detected by PCR/bi-directional sequencing.

3. Testing of Contrived Clinical Specimens

Some respiratory pathogens are so rare that both the prospective and archived specimen collection efforts were insufficient to demonstrate the clinical performance. To supplement the prospective clinical study and retrospective archived study 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 contrived positive samples, were tested. The results of the BioCode RPP testing are presented in Table 42 below.

Table 42: BioCode RPP Performance Testing Contrived Specimens

Pathogen	Source	Strain/Isolate	Concentration (xLoD)	PPA (%)	95% CI	NPA (%)	95% CI
Chlamydia pneumoniae	ATCC 53592	AR-39	2	9/9 (100%)	70.1%, 100%	60/60 (100%)	94.0%, 100%
			10	5/5 (100%)	56.6%, 100%		
			100	4/4 (100%)	51.0%, 100%		
	ATCC VR- 1360	CM-1	2	8/8 (100%)	67.6%, 100%		
			10	5/5 (100%)	56.6%, 100%		
			100	3/3 (100%)	43.8%, 100%		
	ATCC VR- 1310	CWL-029	2	8/8 (100%)	67.6%, 100%		
			10	5/5 (100%)	56.6%, 100%		
			100	3/3 (100%)	43.8%, 100%		
	Combined			50/50 (100%)	92.9%, 100%		
Pathogen	**Source**	**Strain/Isolate**	**Concentration (xLoD)**	**PPA (%)**	**95% CI**	**NPA (%)**	**95% CI**
Influenza A/H1N1	Zeptometrix 0810036CF	A/New Caledonia/20/99	2	5/5 (100%)	56.6%, 100%	60/60 (100%)	94.0%, 100%
			10	3/3 (100%)	43.8%, 100%		

			100	3/3 (100%)	43.8%, 100%		
	Zeptometrix 0810247CF	A/Taiwan/42/06	2	5/5 (100%)	56.6%, 100%		
			10	3/3 (100%)	43.8%, 100%		
			100	2/2 (100%)	34.2%, 100%		
	Zeptometrix 0810246CF	Singapore/63/04	2	5/5 (100%)	56.6%, 100%		
			10	2/2 (100%)	34.2%, 100%		
			100	2/2 (100%)	34.2%, 100%		
	Virapur	A/Denver/1/1957	2	5/5 (100%)	56.6%, 100%		
			10	2/2 (100%)	34.2%, 100%		
			100	2/2 (100%)	34.2%, 100%		
	ATCC VR-219	A/NWS/33	2	5/5 (100%)	56.6%, 100%		
			10	3/3 (100%)	43.8%, 100%		
			100	3/3 (100%)	43.8%, 100%		
	Combined			50/50 (100%)	92.9%, 100%		

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

In the BioCode RPP prospective clinical study, a total of 2647 NPS eluted in VTM or UTM specimens were evaluable by the BioCode RPP. The number and percentage of positive cases per site (Table 43) and per age group (Table 44), as determined by BioCode RPP, are presented below.

Table 43: BioCode RPP Expected Values by Specimen Collection Site

Pathogen	Overall (n=2647)		Site 1 (n=529)		Site 2 (n=419)		Site 3 (n=599)		Site 4 (n=550)		Site 5 (n=550)	
	N	Expected value	N	Expected value	N	Expected value	N	Expected value	N	Expected value	N	Expected value
Adenovirus	109	4.1%	8	1.5%	27	6.4%	21	3.5%	17	3.1%	36	6.5%
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%
Influenza A/H1pdm09	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%
Parainfluenza virus 1	15	0.6%	4	0.8%	0	0%	11	1.8%	0	0%	0	0%
Parainfluenza virus 2	13	0.5%	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%	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%

Pathogen	Overall (n=2647)		Site 1 (n=529)		Site 2 (n=419)		Site 3 (n=599)		Site 4 (n=550)		Site 5 (n=550)	
	N	Expected value	N	Expected value	N	Expected value	N	Expected value	N	Expected value	N	Expected value
Bordetella Pertussis	21	0.8%	0	0%	19	4.5%	1	0.2%	0	0%	1	0.2%
Chlamydophila pneumoniae	5	0.2%	0	0%	3	0.7%	0	0%	1	0.2%	1	0.2%
Mycoplasma pneumoniae	38	1.4%	4	0.8%	15	3.6%	5	0.8%	3	0.5%	11	2.0%

Table 44: BioCode RPP Expected Values by Age Group

Pathogen	Overall (n=2647)		≤ 5 Years (n=1004)		6-21 Years (n=609)		22-59 Years (n=531)		60+ Years (n=503)	
	N	Expected value	N	Expected value	N	Expected value	N	Expected value	N	Expected value
Adenovirus	109	4.1%	80	8.0%	20	3.3%	5	0.9%	4	0.8%
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%
Influenza A/H1pdm09	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%
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%
Parainfluenza virus 3	135	5.1%	74	7.4%	21	3.4%	21	4.0%	19	3.8%
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%
Bordetella Pertussis	21	0.8%	8	0.8%	12	2.0%	0	0%	1	0.2%
Chlamydophila pneumoniae	5	0.2%	1	0.1%	3	0.5%	1	0.2%	0	0%
Mycoplasma pneumoniae	38	1.4%	9	0.9%	21	3.4%	6	1.1%	2	0.4%

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

VIII Proposed Labeling:

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

IX Conclusion:

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