BioCode®

Respiratory Pathogen Panel (RPP)

Package Insert

Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests

Applied BioCode

Barcoded Magnetic Beads | Highly Multiplex Assays

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NAME AND INTENDED USE

BioCode® Respiratory Pathogen Panel (RPP)

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

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

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

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

Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioCode® RPP cannot differentiate them. A positive BioCode® RPP Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is required. The BioCode® RPP detects Human Rhinovirus/Enterovirus with reduced sensitivity. If a more accurate HRV/EV result is required, it is recommended that specimens found to be negative for Human Rhinovirus/Enterovirus, after examination using BioCode® RPP, be confirmed by an alternate method (e.g. FDA cleared molecular tests).

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

SUMMARY AND EXPLANATION OF THE TEST

Acute respiratory infections (ARIs) are viral or bacterial infections that arise in the upper or lower respiratory systems. The World Health Organization (WHO) estimates that young children experience four to eight ARIs each year, with ARIs causing around one third of all deaths in children under the age of five.[1] Even for milder illnesses such as the common cold, the estimated economic impact in the US of non-influenza virus respiratory tract infections is upwards of $40 billion annually when accounting for missed work and school days on top of medical treatment.[2] Some respiratory pathogens cause annual epidemics during a particular season and are tracked by the Centers for Disease Control and Prevention (CDC) in the United States, the European Centre for Disease Prevention and Control (ECDC), and the WHO. The BioCode® Respiratory Pathogen Panel (RPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of 17 respiratory pathogens (see table below) collected via nasopharyngeal swab. The panel is designed to be used with the BioCode® MDx-3000 high throughput system. Test results from the BioCode® RPP are available in about 5 hours.

Table. Pathogen targets included in BioCode® RPP

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

Summary of Detected Organisms

Viruses

Influenza A & B

Influenza viruses are negative-sense single-strand RNA orthomyxoviruses. Influenza A and B are the two major subtypes that cause annual flu epidemics in humans. Common flu symptoms include fever, chills, cough, sore throat, nasal congestion and runny nose, body aches, headaches, and fatigue, with young children sometimes experiencing vomiting and diarrhea. Influenza A subtypes are identified based on antigenic differences in two glycoproteins: hemagglutinin and neuraminidase. Genetic reassortment is responsible for the emergence of novel influenza A strains; notably, a new strain of H1N1 was responsible for the 2009 “swine flu” pandemic. Influenza B has only one serotype and two known lineages – B/Yamagata and B/Victoria – with significant Influenza B epidemics occurring about every 2-3 years.[3] The BioCode® RPP detects and differentiates flu A subtype H1, flu A subtype H1 2009 pandemic (pdm), flu A subtype H3, and flu B.

The flu is spread by droplets when an infected individual coughs, sneezes, or talks.[4] The virus may be present in the body for up to two days before a patient notices any symptoms, and most adults are able to infect others from the day before symptoms arise up to about a week after symptoms develop.[4] Importantly, some individuals infected with a flu virus may be asymptomatic; these people are still capable of spreading the virus to others.[4] A handful of antiviral drugs are available to treat the flu.[4] While the flu circulates year-round in the United States, cases spike in the fall and winter months, with flu season usually beginning in October and lasting as late as May.[4] The CDC closely tracks the number of respiratory specimens that test positive for influenza each flu season and publishes their findings online via FluView and FluView Interactive. Similarly, the European Centre for Disease Prevention and Control tracks flu cases in Europe and publishes weekly reports through the European Influenza Surveillance Network (EISN).[5, 6] The WHO has tracked global data on flu cases and the predominant subtypes effecting different countries since the 1950s and uses their data to issue recommendations on flu vaccine compositions.[7] The WHO estimates that each year, flu epidemics are responsible for between 3 and 5 million serious illnesses and up to 650,000 deaths worldwide, with most deaths in industrialized nations reported in adults over the age of 65 and the vast majority of deaths in children under the age of 5 occurring in developing countries.[8] The WHO recommends annual influenza vaccinations for young children, the elderly, pregnant women, healthcare workers, and individuals with chronic medical conditions.[8]

Parainfluenza Viruses 1-4

Human parainfluenza viruses are negative-sense single-strand RNA viruses from the *Paramyxoviridae* family. Four major serotypes cause respiratory illness in humans and are detected and differentiated by the BioCode® RPP: human parainfluenza virus type 1 (PIV-1), human parainfluenza virus type 2 (PIV-2), human parainfluenza virus type 3 (PIV-3), and human parainfluenza virus type 4 (PIV-4). These viruses were distinguished from influenza viruses in the 1950s.[9] Like influenza, parainfluenza viruses are spread when an infected person coughs or sneezes but can also be transmitted through personal contact such as shaking hands or when someone touches a contaminated surface and then touches their mouth, nose, or eyes.[10] Parainfluenza viruses can infect both the lower and upper respiratory tracts with upper respiratory symptoms including fever, rhinorrhea, and cough, and lower respiratory symptoms including croup, bronchitis, bronchiolitis, and pneumonia.[10] The CDC monitors parainfluenza cases in the US via the National Respiratory and Enteric Virus Surveillance System (NREVSS). In the US, PIV-1 and PIV-2 infections usually spike in the fall while PIV-3 is more frequently detected during the spring and early summer, whereas PIV-4 is less common and does not have a well-characterized season.[10] PIV1-3 are second only to RSV in the number of US pediatric hospitalizations they cause due to lower respiratory tract infections.[9, 11] Currently there is no available vaccine for any of the parainfluenza viruses.[10]

Respiratory Syncytial Virus

Human respiratory syncytial virus (RSV), also known as human orthopneumovirus, is a negative-sense single-strand RNA virus divided into subgroups A and B. The BioCode® RPP has one assay that detects for both RSV A and RSV B. Like other respiratory viruses, RSV is spread through droplets expelled when an infected person coughs or sneezes.[12] The virus generally causes mild cold-like symptoms such as runny nose, sneezing, and coughing, but may cause serious illness in young children and the elderly.[12] RSV is the leading cause of severe respiratory illness in infants and young children, with 2.1 million outpatient visits and over 50,000 hospitalizations in children under five in the US annually.[12, 13] Worldwide it is estimated that between 66,000 and 199,000 children under the age of five die each year from RSV.[14] Among adults older than 65 in the US, RSV is responsible for an average of 177,000 hospitalizations and 14,000 deaths per year.[15] In the US, RSV cases spike during the fall, winter, and spring; different countries may experience RSV seasons at varying times and durations based on climate and latitude.[12, 13] There is no RSV vaccine currently available, although the monoclonal antibody palivizumab can be administered in monthly doses

to high-risk individuals such as infants born prematurely to prevent RSV infection during the peak season.[12, 13] The CDC monitors RSV cases in the US via the National Respiratory and Enteric Virus Surveillance System (NREVSS) while the European Influenza Surveillance Network (EISN) tracks data on RSV and other influenza-like illnesses in Europe.

Human Metapneumovirus

Human metapneumovirus (hMPV) was discovered in 2001 and is a member of the *Pneumoviridae* virus family along with respiratory syncytial virus.[16, 17] The BioCode® RPP has two assays that both detect for hMPV subtypes A and B. Metapneumovirus can cause mild upper and lower respiratory illnesses with symptoms such as cough, fever, nasal congestion, and shortness of breath, but these symptoms may progress to bronchitis or pneumonia, especially in young children, elderly adults, and individuals with weakened immune systems.[17] The virus can be spread from coughing, sneezing, close personal contact with an infected individual, or touching contaminated surfaces.[17] An estimated 20,000 hospitalizations in children under five years old are associated with hMPV in the US each year.[18] The virus peaks during the late winter and spring and typically circulates simultaneously with RSV and influenza.[17, 19] The CDC's National Respiratory and Enteric Virus Surveillance System (NREVSS) tracks hMPV cases in the US while European outbreaks are reported in the journal *Euro Surveillance*.[20, 21]

Human Rhinovirus/Enterovirus

Rhinovirus (HRV) and enterovirus (EV) both belong to the *Picornaviridae* family. Rhinoviruses are subdivided into three distinct groups – A, B, and C – with over 100 serotypes.[22] HRV is the most frequent cause of the common cold and accounts for the majority of upper respiratory infections in the spring, summer, and fall while influenza and RSV dominate in the winter months.[22, 23] Classic symptoms of the common cold are typically milder than the flu and include runny nose, sore throat, coughing, sneezing, headaches, and body aches lasting 7-10 days on average.[4, 23] The CDC estimates that adults experience 2-3 colds per year, while young children can become infected more often.[23] Non-polio enteroviruses such as enterovirus D68 (EV-D68) can also cause the common cold.[24] EV-D68 infections tend to peak in the summer and fall, and children with asthma have a higher risk of developing a more serious respiratory illness if infected with EV-D68.[24] While once considered rare, a nationwide outbreak of EV-D68 occurred in the US in 2014 that mainly affected children.[24, 25] There are currently no antiviral treatments available for HRV or EV.[22, 23] The BioCode® RPP has one assay that detects rhinoviruses and enteroviruses without differentiation.

Coronavirus

Coronaviruses belong to the *Coronaviridae* virus family and contain positive-sense single-strand RNA. Coronaviruses OC43, HKU1, NL63, and 229E can all cause mild to moderate upper respiratory illness such as the common cold while infants, the elderly, and patients with weakened immune systems or cardiopulmonary disease are at a greater risk to develop lower respiratory infections including pneumonia and bronchitis.[26] Two other important coronaviruses, MERS-CoV and SARS-CoV, are responsible for more severe respiratory diseases and have caused major outbreaks in recent years.[26] As with other respiratory pathogens, coronaviruses are transmitted through droplets when an infected person coughs or sneezes.[26] In the US, coronavirus infections can occur year-round but are most common during the fall and winter months.[26] The CDC tracks coronavirus cases with the National Respiratory and Enteric Virus Surveillance System (NREVSS) while the ECDC mainly tracks MERS-CoV and SARS-CoV.[26, 27, 28] As with colds caused by HRV, there is no specific antiviral treatment available for coronavirus and most infections are self-limiting.[26] The BioCode® RPP has 4 assays for detection of coronaviruses OC43, HKU1, NL63, and 229E without differential reporting of subtypes. The BioCode® RPP coronavirus assays do not detect MERS-CoV and SARS-CoV.

Adenovirus

Adenoviruses are non-enveloped icosahedral viruses from the *Adenoviridae* family and contain double-strand DNA. Adenoviruses are ubiquitous in the environment and are resistant to chemical and physical damage. These viruses are classified into types A-G and can cause a host of diseases including respiratory infections, gastroenteritis, and conjunctivitis.[29] Adenovirus types 3, 4, 7, and 14 are most often associated with respiratory illnesses such as the common cold, pneumonia, croup, and bronchitis.[29] The US military offers a vaccine to new recruits covering adenovirus types 4 and 7 which are notorious for causing respiratory infections in cadets during basic training, but this vaccine is not available to the general public.[30] The CDC collects data on adenovirus cases through the National Adenovirus Type Reporting System (NATRS) and specifically respiratory illnesses caused by adenovirus through the National Respiratory and Enteric Virus Surveillance System (NREVSS). The BioCode® RPP detects various types of adenoviruses without differentiation

Bacterial Pathogens

Mycoplasma pneumoniae

Mycoplasma pneumoniae are small bacteria from the class Mollicutes that do not feature a peptidoglycan cell wall. *M. pneumoniae* causes tracheobronchitis which is characterized by sore throat, cough, fever, and fatigue, as well as a mild form of pneumonia often referred to as atypical pneumonia or walking pneumonia.[31] These diseases peak in the summer and early fall and are spread when an infected individual coughs or sneezes, spreading droplets that contain the bacteria.[31, 32] *M. pneumoniae* has a relatively long incubation period of 1 to 4 weeks.[32] Mild *M. pneumoniae* infections are usually self-limiting while more serious or prolonged illnesses are treated with antibiotics, although the absences of a cell wall in *M. pneumoniae* renders many common antibiotics ineffective.[33] Children, members of the military housed in barracks, elderly individuals in nursing homes, and young adults living in college residence halls are the most at risk for developing *M. pneumoniae* infections.[31] The BioCode® RPP has one assay for detection of *M. pneumoniae.*

Chlamydia pneumoniae

Chlamydia pneumoniae are obligate intracellular bacteria of the bacterial family *Chlamydiaceae* that can only replicate after they have been taken up by another cell via phagocytosis. This bacterium causes both upper and lower respiratory infections; upper respiratory symptoms include runny nose, congestion, headache, cough, sore throat, and hoarseness or loss of voice, while lower respiratory infections include bronchitis and atypical pneumonia.[34] Some *C. pneumoniae* infections are asymptomatic.[34] Like *Mycoplasma pneumoniae*, *C. pneumoniae* is spread from coughing or sneezing droplets containing the bacteria and has a relatively long incubation period with symptoms appearing 3-4 weeks after exposure.[34] Most *C. pneumoniae* infections are resolved on their own but antibiotics can be used to treat the bacteria.[34] *C. pneumoniae* can be contracted year-round and does not have a particular season.[34] As with *M. pneumoniae*, populations most at risk for developing *C. pneumoniae* infection include children, military personnel, and people living in nursing homes or college dormitories.[34] The BioCode® RPP has one assay for detection of *C. pneumoniae.*

Bordetella pertussis

Bordetella pertussis is a gram-negative, non-motile bacterium responsible for pertussis, also known as whooping cough. The hallmark of pertussis is a paroxysmal cough followed by a “whooping” sound in addition to fever, runny nose, and sometimes vomiting. The disease has about a week-long incubation period and coughing fits can linger for several weeks.[35, 36] Pertussis is most dangerous to infants, with half

of all infected infants requiring hospitalization.[36] To treat pertussis the CDC recommends administering appropriate antibiotics within 3 weeks of suspected exposure.[36] Vaccines against *Bordetella pertussis* are available worldwide, and the WHO estimates that 687,000 deaths were prevented in 2008 due to pertussis vaccination programs. The WHO recommends a three-dose vaccine be administered to infants beginning at 6 weeks of age and for boosters to be given to pregnant women.[35] In the US, pertussis vaccination is administered through the DTaP vaccine given to infants and children under 7 years old and through the Tdap vaccine for older children and adults.[36] The CDC tracks pertussis in the US through the National Notifiable Diseases Surveillance System (NNDSS) while the ECDC publishes an annual epidemiological report detailing pertussis cases in Europe. The BioCode® RPP has one assay for detection of *B. pertussis.*

PRINCIPLE OF PROCEDURE

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 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 NucliSENS® easyMAG® or MagNA Pure 96 automated systems according to the manufacturer provided protocol.

Overview of a BioCode® MDx-3000 Run

1. **Reverse Transcription and Multiplex PCR** – Since targets of the BioCode® RPP include RNA viruses, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a freshly prepared reaction 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.
2. **Dispensing BMB-Probe Mix** – Towards the end of PCR amplification, the robotic head dispenses BMB-Probe mix into the designated reaction wells of the capture plate using disposable pipette tips.
3. **PCR Product Transfer** – After PCR amplification is completed, the robotic head pierces the foil seal with disposable pipette tips and transfers PCR products into corresponding wells of the capture plate.
4. **Target Capture** – Amplified PCR products labeled with biotin are captured at a defined temperature by target-specific probes that are covalently coupled to designated 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 (BMB) for each pathogen and the internal control.
5. **Signal Generation** – After washing off unbound PCR products and unused primers, a streptavidin-phycoerythrin (SA-PE) conjugate is automatically added to the reaction by the robot. High affinity binding between biotin and streptavidin ensures that captured PCR products with the biotin moiety are labeled with phycoerythrin in close proximity to the BMBs.
6. **Optical Detection** – Optical detection is performed for each reaction well of the capture plate, an optically clear, flat-bottom microtiter plate. After washing off unbound SA-PE, excitation of the fluorophore at the designated wavelength emits fluorescence signal from BMBs tagged with SA-PE conjugates. Each reaction well is imaged at a specific emission wavelength for fluorescent signal and under bright field for identifying the barcode patterns (decoding).

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

MATERIALS REQUIRED

Materials Provided With Each Kit

- BioCode® Respiratory Pathogen Panel Kit (Applied BioCode 63-R0001)
  - BioCode® Master Mix A (store at -20°C, after thaw store at 4°C for up to 30 days)
  - BioCode® RPP Primer Mix (store at -20°C, after thaw store at 4°C for up to 30 days)
  - BioCode® RT Mix (store at -20°C)
  - BioCode® RNA IC2 (store at -20°C, after thaw store at 4°C for up to 30 days)
  - BioCode® RPP BMB-Probe Mix (store at -20°C, after thaw vortex for 30 sec, store at 4°C up to 90 days)

Materials Required But Not Provided With Each Kit

- BioCode® SA-PE Mix (single use; protect from light; store at 4°C. **Do Not Freeze**) (Applied BioCode 63-S0001)
- BioCode® Buffer A (store at room temp) (Applied BioCode 44-B0003)
- BioCode® MDx-3000
- BioCode® MDx-3000 Consumables
  - Reagent Reservoirs (Integra 4332, 01-R0005)
  - Waste Bin and Lid (Applied BioCode 01-W0105 and 01-W0104))
  - 20 µL pipette tips (Beckman 717256, 01-P0006)
  - 250 µL pipette tips (Beckman 717252, 01-P0007)
  - Bio-Rad 96-well hard shell plate 0.1 mL (HSL9601, 01-P0011)
  - PCR Adhesive Foil (Thermo Fisher Scientific AB-0626 or Eppendorf 0030127790, 01-P0012)
  - Microtiter plate (Greiner Bio-One 655101, 01-P0009)
  - Microtiter plate lid (Nunc 5500, 01-P0010)
- NucliSENS® easyMAG® (BioMérieux) or MagNA Pure 96 (Roche) Extraction System
- easyMAG® supplies for extraction
  - Lysis Buffer
  - Buffer 1
  - Buffer 2
  - Buffer 3
  - Magnetic silica
  - Nuclease-free water
  - Consumables
  - ELISA strip plate
- MagNA Pure 96 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 extractions Freezer (manual defrost) at -10 to -30°C
- Freezer (manual defrost) at -60 to -90°C
- Refrigerator at 2 to 8°C

WARNINGS AND PRECAUTIONS

General Precautions

1. For *In Vitro* Diagnostic Use only.
2. For Prescription Use Only.
3. Results should be interpreted in combination with the patient's signs and symptoms and results from other diagnostic tests by a trained healthcare professional.
4. The BioCode® Respiratory Pathogen Panel is for use with the BioCode® MDx-3000 instrument only.

Precaution Related to Public Health Reporting

Local, state, and federal rules and regulations for notification of reportable diseases are continually updated and include a number of organisms that are important for surveillance and outbreak investigations. Laboratories are responsible for following their state and/or local rules pertaining to reportable pathogens and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

Laboratory Precautions

1. Perform the protocol as described in this package insert. Deviations from this protocol may produce erroneous results.
2. The BioCode® RPP should be performed in clearly defined work areas moving in one direction from pre-amplification areas to the amplification/detection area to reduce potential for contamination.
   a. Begin with specimen preparation and reagent preparation before moving to amplification/detection.
   b. Use dedicated equipment and supplies for each area (including personal protective equipment, such as lab coats and disposable gloves).
   c. Clean work areas with 10% bleach or similar disinfectant followed by water before and after assay preparation.
3. A negative control must be tested for each run. If multiple lots are assayed at the same time, a negative control must be assayed for each lot.
4. Do not use reagents past the expiration date. Do not mix reagents or interchange kit components from different kit lots. Kit configurations are identified on the Kit outer carton and the Kit Card.
5. Assay setup should be performed at room temperature. Keep Reaction Mix cold using a cooling block during formulation and loading of amplification plate.

Safety Precautions

1. Follow universal safety procedures. All patient specimens should be considered potentially infectious and handled accordingly.
2. Dispose of unused kit reagents and specimens according to local, state and federal regulations.
3. Wear appropriate personal protective equipment including, but not limited to, lab coats, gloves, and protective eyewear. Change gloves often.
4. Do not pipette by mouth.
5. BioCode® RT Mix is classified as an irritant. See SDS for details.

REAGENT STORAGE, HANDLING AND STABILITY

1. Store the RPP kit components frozen (-20°C) prior to use.
2. Store RT Mix frozen (-20°C) except during use.
3. Once thawed, store Master Mix, Primer Mix, and RNA IC2 refrigerated (2-8°C) for up to 30 days.
4. Once thawed, store BMB-Probe Mix refrigerated (2-8°C) for up to 90 days.
5. SA-PE mix is for single use only. Store refrigerated (2-8°C). Protect from light. **DO NOT FREEZE.**
6. Store the Buffer A at room temperature (15-25°C).
7. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
8. Always check the expiration date and do not use reagents beyond the expiration date printed.
9. Once RT-PCR reaction mix is prepared, the test run should be started as soon as possible (within 60 minutes).
10. Remove BMB-Probe Mix from MDx-3000 once the run is completed and store refrigerated (2-8°C).

SAMPLE REQUIREMENTS

This section describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Nasopharyngeal Swab (NPS) should be collected using flocked swabs according to standard technique and immediately placed in 1 - 3 mL of VTM or UTM. Samples should be tested as soon as possible. They may be stored at the following conditions:

- Room temperature for 8 hours
- 2 - 8°C for 7 days
- <-60°C for up to 90 days

Minimum Sample Volume - 200 µL of sample is required for testing.

PROCEDURE

Refer to the BioCode® MDx-3000 Operator's Manual for more detail and pictorial representations of the BioCode® MDx-3000 set up instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling specimens and reagents. Once PCR reagents are prepared and sample is added to PCR plate, it should be promptly transferred to the instrument to start the run. After the run is complete, the PCR plate and capture plate should be sealed and discarded.

Extraction Methods

Note: It is strongly recommended that sample preparation be performed in a biosafety cabinet with gloves and appropriate personal protective equipment (PPE).

easyMAG® Extraction

1. Pipet 10 µL RNA IC2 into each well of the easyMAG® cartridge
2. Transfer 200 µL of NPS specimen or Control into easyMAG® cartridge and load into easyMAG®
3. Perform Protocol: **Generic 2.0.1, volume 0.200 mL, Eluate: 50.0 µL, Sample Type: Primary, Matrix: Other**
   - **3.1** Perform 10 min on-board incubation
   - **3.2** When prompted, add magnetic silica
     - 3.2.1 Combine 550 µL nuclease-free water and 550 µL magnetic silica mix in one 1.5 mL tube per easyMAG® cartridge
     - 3.2.2 Mix thoroughly and dispense 125 µL into each well of an 8-well ELISA strip per easyMAG® cartridge.
     - 3.2.3 Add 100 µL to each easyMAG® cartridge well and mix thoroughly
   - **3.3** Start remainder of run

MagNA Pure 96 Extraction

1. Pipet 10 µL RNA IC2 into each well of the MagNA Pure 96 processing cartridge (Be careful to pipet directly to the bottom of each well in the cartridge and not produce bubbles. Liquid on the side of the well and bubbles will lead to incorrect volume sensing and the extraction will be aborted.)
2. Transfer 200 µL of NPS specimen or Control into the MagNA Pure 96 processing cartridge (Be careful to pipet directly to the bottom of each well in the cartridge and not produce bubbles. Liquid on the side of the well and bubbles will lead to incorrect volume sensing and the extraction will be aborted.)
3. Perform Protocol: **Pathogen Universal 200 3.1 for MagNA Pure Kit: DNA/Viral NA SV 2.0. Volume: 200 µL, Eluate: 50 µL.**

Nucleic Acid Storage Conditions

Transfer sample extracts from the cartridge into PCR grade micro-tubes, strips or plates and store samples in a 2-8°C refrigerator if testing within 12 hours. Store at -60°C or below if testing cannot be completed within 12 hours of extraction. Extracted nucleic acids may be stored at -60°C or below for up to 90 days.

BioCode® RPP Set Up

Note: Prepare the PCR Plate in a dedicated reaction mix prep area.

1. Thaw Primer Mix, Master Mix and BMB-Probe Mix at room temperature. Perform a quick vortex (2-3 seconds) and centrifuge to collect reagents at the bottom of the tube.
2. Prepare the reaction mix in a polypropylene microcentrifuge tube as described below:

Table. Reaction Mix Formulation

Component	Reaction Mix Volume (µL) per reaction	Reaction Mix Volume (µL) per 10 reactions
BioCode® Master Mix A	10.0 µL	100 µL
BioCode® RPP Primer Mix	9.5 µL	95 µL
BioCode® RT Mix	0.5 µL	5 µL
Reaction Mix Volume (µL)	**20 µL**	**200 µL**

3. Mix reaction mix by pipetting up and down 8 to 10 times and centrifuge to collect contents at the bottom of the tube. Store at 2-8°C or on a cooling block until ready to set up PCR (not to exceed one hour). Do NOT vortex reaction mix.
4. Pipette 20 µL of reaction mix into appropriate wells of a 96-well plate.
5. Pipette 5 µL of each extracted sample into the wells.
6. Pipette 5 µL extracted negative control into the NC well.
7. Seal plate with pierceable foil. Store at 2-8°C or on a cooling block until ready to load onto the BioCode® MDx-3000 (not to exceed one hour from the time the reaction mix is prepared).
8. Briefly centrifuge plate to collect samples at the bottom of the plate.
9. Load plate onto BioCode® MDx-3000.
10. Vortex the thawed room temperature BMB-Probe Mix for 30 seconds at high speed and load onto the BioCode® MDx-3000. **(Note: Precipitates may appear at cold temperatures. If precipitates are present, allow the BMB-Probe Mix to warm to room temperature and vortex for an additional 30 seconds.)**
11. Load reagents and consumables as prompted by graphic user interface and run BioCode® Respiratory Pathogen Panel Protocol.

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. The 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.

External Negative Controls

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

Table. Criteria for Valid 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

Each laboratory should establish its own Quality Control (QC) ranges and frequency of QC testing based on applicable local laws, regulations and good laboratory practices.

External positive controls can be well characterized clinical samples or positive strains. Controls can be single analytes or pooled specimens. The positive controls should go through all processing steps (extractions, amplification, and detection). It is recommended that at least one positive control be included for each plate/kit lot on a rotating schedule. Wells identified as 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 below). The software will not suppress results based on positive control results. If a positive control does not perform as expected, the user should review all samples in that batch to determine if results should be reported.

Table. Criteria for Valid 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 prior to release.
Positive Control	Unexpected Target Detected	N/A	Report will indicate positive control is Invalid. User should review results prior to release.

Internal Control

An RNA Internal Control (RNA IC2: 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 results may be reported in the absence of RNA IC detection. However, the BioCode® MDx-3000 software will suppress negative results for any wells with invalid RNA IC results (see table).

Table. 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. Repeat/reflex testing.
Not Detected	Not Detected	Well status: Invalid. Not Detected results suppressed by software. Repeat/reflex testing.

Lack of RNA IC signal may indicate sample-associated inhibition or reagent/instrumentation issues. Samples suspected of being inhibitory should be repeated from extraction. If reagent or instrument issues are suspected specimens may be repeated from stored nucleic acid extracts.

Target Pathogen Interpretation

Fluorescent signals from BMBs with the same barcode are sorted and the median fluorescence intensity (MFI) is calculated for each analyte. The assays are considered "Detected" by comparing the MFI to a validated assay cutoff. For many targets in the BioCode® RPP, the target is considered to be detected if a single corresponding assay is positive. For example, Respiratory Syncytial Virus will have a result of "Respiratory Syncytial Virus Detected" if target specific MFI is at or above the threshold. The following targets are detected using a single assay: Respiratory Syncytial Virus, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Rhinovirus/Enterovirus, *Bordetella pertussis*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*.

Table. Single target Assay names with corresponding results.

Assay Name	Assay Result	Report Result
RSV	Detected	Respiratory Syncytial Virus Detected
PIV1	Detected	Parainfluenza Virus 1 Detected
PIV2	Detected	Parainfluenza Virus 2 Detected
PIV3	Detected	Parainfluenza Virus 3 Detected
PIV4	Detected	Parainfluenza Virus 4 Detected
HRV	Detected	Rhinovirus/Enterovirus Detected
BP	Detected	*Bordetella pertussis* Detected
MPN	Detected	*Mycoplasma pneumoniae* Detected
CPN	Detected	*Chlamydia pneumoniae* Detected

In contrast, the test results for several targets rely on the combination of multiple assays. These include Influenza B, Human Metapneumovirus, Adenovirus and Coronavirus. Each assay is compared to the set threshold and if any of the assays are detected the report result will be "Detected". The tables below outlines possible assay results and the corresponding test reports.

Table. Possible Assay results and corresponding report results for targets with 2 assays.

Assay Name/Result	Assay Name/Result	Report Result
FluB1 / Not Detected	FluB2 / Not Detected	Influenza B Not Detected
FluB1 / Detected	FluB2 / Any Result	Influenza B Detected
FluB1 / Any Result	FluB2 / Detected	Influenza B Detected
HMPV1 / Not Detected	HMPV2 / Not Detected	Human Metapneumovirus Not Detected
HMPV1 / Detected	HMPV2 / Any Result	Human Metapneumovirus Detected
HMPV1 / Any Result	HMPV2 / Detected	Human Metapneumovirus Detected
ADV1 / Not Detected	ADV2 / Not Detected	Adenovirus Not Detected
ADV1 / Detected	ADV2 / Any Result	Adenovirus Detected
ADV1 / Any Result	ADV2 / Detected	Adenovirus Detected

Table. Possible Assay results and corresponding report results for Coronavirus (4 assays).

Assay 1 (229E)	Assay 2 (HKU1)	Assay 3 (NL63)	Assay 4 (OC43)	Report Result
Not Detected	Not Detected	Not Detected	Not Detected	Coronavirus Not Detected
Detected	Any Result	Any Result	Any Result	Coronavirus Detected
Any Result	Detected	Any Result	Any Result	Coronavirus Detected
Any Result	Any Result	Detected	Any Result	Coronavirus Detected
Any Result	Any Result	Any Result	Detected	Coronavirus Detected

In addition, samples detected by the Influenza A assay can be further differentiated by Influenza A hemagglutinin subtype assays specific for: H1, H1 2009pdm, and H3. The panel contains one assay designed to detect Influenza A (FluA) and three HA subtyping assays (FluA/H1, FluA/H1 2009pdm, and FluA/H3). The table below outlines possible assay results and the corresponding test reports. Indeterminate or Influenza A only (no subtype detected) results should be retested.

Table. Possible Assay results and corresponding report results for Influenza A and HA subtyping assays

Target / Assay / BioCode® RPP Result	FluA	FluA/H1	FluA/H1 pdm09	FluA/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/H3 Detected	Detected	Not Detected	Not Detected	Detected	Report result
Influenza 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)
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 retest 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.

Note: As polymicrobial results with four or more distinct targets in a single sample are unusual based upon data from the prospective clinical study, confirmation of this result is recommended to rule out any unexpected error, either caused by the user's handling of the sample or the test system. Polymicrobial results of four or more targets were detected in less than 0.11% (3/2649) of the BioCode® RPP prospective study specimens.

BioCode® RPP Test Report

The analyzed BioCode® MDx-3000 results are displayed in two report formats: Run Report for the entire run including multiple specimens, or 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. Refer to operator manual for more details and examples of the BioCode® MDx-3000 reports.

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 are: Detected, Not detected, Indeterminate (for Influenza A only), Invalid, or N/A (if not ordered).

The Sample report displays results for a single well (specimen/control). In addition to results for each target, 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 and expiration dates. The headers also include sections for Run Status and External Controls status. The Run Status section will specify if the run is Incomplete, Valid or Invalid based on the Negative Control results for the specific run/kit lot. The External Controls section indicates the results for the negative controls (Valid or Invalid) and Positive Controls (Valid, Invalid, or N/A if not assayed). The Run Status and Controls sections should be reviewed prior to review of target results. In addition to these summaries, the software will also mask results in the detailed tabular sections based on plate and well validity requirements (see interpretation of results for details).

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

LIMITATIONS OF THE PROCEDURE

- For prescription use only.
- The BioCode® Respiratory Pathogen Panel is for use with the BioCode® MDx-3000 instrument only.
- Results of this test should be interpreted by a trained clinician in conjunction with clinical history, epidemiological data and any other laboratory data.
- If four or more distinct pathogens are detected in a specimen, retesting the specimen is recommended to confirm the polymicrobial result.
- This assay is qualitative and does not provide a quantitative value for the pathogen(s) present in the sample.
- The performance of the BioCode® RPP has been validated with nasopharyngeal swab (NPS) specimens in VTM or UTM only. It has not been validated for other specimen types or samples stored in other transport media.
- The performance of this test has not been established for patients without signs of symptoms of respiratory infection.
- The performance of the BioCode® RPP is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled specimens. The internal control (RNA IC2) will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- Negative results do not exclude the possibility of infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, or an infection caused by a pathogen not detected by the panel. Test results may also be affected by concurrent antimicrobial therapy or levels of pathogen in the sample that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other 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 is not detected by a nasopharyngeal swab specimen.
- Nucleic acid may persist independently of a pathogen's viability or pathogens may be asymptomatically carried. Therefore, a positive result does not necessarily indicate the presence of viable pathogens or that the pathogen is the causative agent for the clinical symptoms.
- There is a risk of false positive results due to cross-contamination by target organisms, their nucleic acids or amplified product. Particular attention should be given to the Laboratory Precautions noted under the *Warnings and Precautions* section above.
- There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge are possible.
- The performance of the test has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the *Interference* section below could lead to erroneous results.

- The performance of this test has not been established for monitoring treatment of infection with any of the panel targets.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The performance of the BioCode® RPP has not been established in individuals who received the influenza vaccine. Recent administration of the nasal influenza vaccine may cause false positive results for Influenza A and/or Influenza B.
- The BioCode® RPP Influenza A subtyping assay is based on the hemagglutinin gene. This assay does not differentiate based on the Influenza A neuraminidase gene.
- The effect of antibiotic treatment on test performance has not been evaluated.
- The performance of this test has not been established for screening of blood or blood products.
- Clinical performance of this test was established when Influenza A/H3 and Influenza A/H1pdm09 were the predominant Influenza A virus in circulation. When other Influenza A viruses are emerging, performance may vary.
- Due to the small number of positive specimens collected for certain pathogens during the prospective clinical study, performance characteristics for *Bordetella pertussis* and *Chlamydia pneumoniae* were established primarily with retrospective clinical specimens. Performance characteristics for Influenza A/H1 were established primarily using contrived clinical specimens.
- The BioCode® RPP may not be able to distinguish between existing influenza strains and new variants as they emerge. For example, BioCode® RPP can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal. In addition, based on *in silico* analysis, BioCode® RPP may detect Influenza A H1N2v as Influenza A H1 with reduced sensitivity, but not as Influenza A H1 2009pdm. If variant virus infection is suspected, clinicians should contact their state or local health department to arrange specimen transport and request a timely diagnosis at a state public health laboratory.
- Positive and negative predictive values are highly dependent on prevalence. False negative results are more likely during peak activity when prevalence of disease is high. False positive results are more likely during periods when prevalence is moderate to low.
- Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioCode® RPP cannot differentiate them. A positive BioCode® RPP Rhinovirus/Enterovirus result should be followed up using an alternate method (e.g., cell culture or sequence analysis) if differentiation is required. The BioCode® RPP detects Human Rhinovirus/Enterovirus with reduced sensitivity. If a more accurate HRV/EV result is required, it is recommended that specimens found to be negative for Human Rhinovirus/Enterovirus after examination using BioCode® RPP be confirmed by an alternate method (e.g. FDA cleared molecular tests).
- Clinical evaluation indicates a lower sensitivity for the detection of Coronaviruses 229E and HKU1. If infection with Coronavirus 229E and/or Coronavirus HKU1 is suspected, negative samples should be confirmed using an alternative method.
- The BioCode® RPP *Bordetella pertussis* assay may amplify IS*481* sequences in *B. bronchiseptica* and *B. holmesii*, generating false positive results.

- 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.
- Flu A (no subtype detected) test results may occur from sequence variants in the region targeted by the Flu A HA subtyping assays. BioCode® RPP will likely report a Flu A (no subtype detected) result when testing patient samples with a Flu A/H3 strain that harbors a mismatch (similar to A/Kansas/14/2017 strain) at lower concentrations. Although estimated prevalence of a sequence variant based solely on *in silico* analysis may not accurately reflect the actual prevalence of the sequence variant in circulation during an influenza season, based on an *in silico* analysis, of all the Flu A/H3 strains isolated in 2019 with published HA sequences, 73.8% of the strains harbor this particular sequence mismatch.

EXPECTED VALUES

In the BioCode® RPP prospective clinical study, a total of 2647 leftover, de-identified NPS eluted in VTM or UTM specimens were evaluable by the BioCode® RPP.

The table below presents the positive results via the BioCode® RPP stratified by age group.

Table. Expected values (as determined by BioCode® RPP) by age group for prospective clinical samples

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

PERFORMANCE CHARACTERISTICS

Clinical Performance

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 Standard of Care (SOC), and were either tested freshly with the BioCode® RPP (i.e., specimens that were stored in a 2-8°C refrigerator for no more than 7 days), or stored frozen and then thawed and tested with the BioCode® RPP at a testing site at a later date (i.e., specimens that were initially stored in a 2-8°C refrigerator but were not able to be tested by the BioCode® RPP within 7 days from specimen collection).

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

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

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

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

The prospective specimens enrolled for evaluation were tested at the five testing sites by trained laboratory personnel. DNA/RNA was extracted using either the BioMérieux 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: Demographics – Prospective Samples

Prospective Study Specimens	
Total Specimens	2649
Gender	**n/N (%)**
Male	1346/2649 (50.8%)
Female	1303/2649 (49.2%)
Age Category	**n/N (%)**
0-5 yrs	1004/2649 (37.9%)
6-21 yrs	609/2649 (23.0%)
22-59 yrs	531/2649 (20.0%)
60+ yrs	505/2649 (19.1%)
Status	**n/N (%)[a]**
Inpatient	1555/2646 (58.8%)
Outpatient	1091/2646 (41.2%)

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

Table: Prospective Sample Type and Test Method Breakdown

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

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

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

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

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

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

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

Target	Storage	(n)	Positive Agreement		Negative Agreement	
			PA (%)	95% CI	NA (%)	95% CI
Adenovirus[a]	Fresh	1400	31/40 (77.5%)	(62.5%, 87.7%)	1340/1360 (98.5%)	(97.7%, 99.0%)
	Frozen	1247	37/38 (97.4%)	(86.5%, 99.5%)	1188/1209 (98.3%)	(97.4%, 98.9%)
	Total	**2647**	**68/78 (87.2%)**	**(78.0%, 92.9%)**	**2528/2569 (98.4%)**	**(97.8%, 98.8%)**
Bordetella pertussis[b]	Fresh	1400	1/1 (100%)	(20.7%, 100%)	1387/1399 (99.1%)	(98.5%, 99.5%)
	Frozen	1247	1/1 (100%)	(20.7%, 100%)	1239/1246 (99.4%)	(98.8%, 99.7%)
	Total	**2647**	**2/2 (100%)**	**(34.2%, 100%)**	**2626/2645 (99.3%)**	**(98.9%, 99.5%)**
Chlamydia pneumoniae[c]	Fresh	1400	2/2 (100%)	(34.2%, 100%)	1397/1398 (99.9%)	(99.6%, 100%)
	Frozen	1247	2/2 (100%)	(34.2%, 100%)	1245/1245 (100%)	(99.7%, 100%)
	Total	**2647**	**4/4 (100%)**	**(51.0%, 100%)**	**2642/2643 (100%)**	**(99.8%, 100%)**
Coronavirus[d]	Fresh	1400	35/50 (70%)	(56.2%, 80.9%)	1338/1350 (99.1%)	(98.5%, 99.5%)
	Frozen	1247	76/83 (91.6%)	(83.6%, 95.9%)	1154/1164 (99.1%)	(98.4%, 99.5%)
	Total	**2647**	**111/133 (83.5%)**	**(76.2%, 88.8%)**	**2492/2514 (99.1%)**	**(98.7%, 99.4%)**
Human Metapneumovirus[e]	Fresh	1400	89/93 (95.7%)	(89.5%, 98.3%)	1299/1307 (99.4%)	(98.8%, 99.7%)
	Frozen	1247	46/49 (93.9%)	(83.5%, 97.9%)	1189/1198 (99.2%)	(98.6%, 99.6%)
	Total	**2647**	**135/142 (95.1%)**	**(90.2%, 97.6%)**	**2488/2505 (99.3%)**	**(98.9%, 99.6%)**

Target	Storage	(n)	Positive Agreement		Negative Agreement	
			PA (%)	95% CI	NA (%)	95% CI
Human Rhinovirus/Enterovirus[f]	Fresh	1400	221/261 (84.7%)	(79.8%, 88.5%)	1119/1139 (98.2%)	(97.3%, 98.9%)
	Frozen	1247	162/213 (76.1%)	(69.9%, 81.3%)	1020/1034 (98.6%)	(97.7%, 99.2%)
	Total	**2647**	**383/474 (80.8%)**	**(77.0%, 84.1%)**	**2139/2173 (98.4%)**	**(97.8%, 98.9%)**
Influenza A[g]	Fresh	1398	115/120 (95.8%)	(90.6%, 98.2%)	1265/1278 (99.0%)	(98.3%, 99.4%)
	Frozen	1244	98/101 (97.0%)	(91.6%, 99.0%)	1131/1143 (99.0%)	(98.2%, 99.4%)
	Total	**2642**	**213/221 (96.4%)**	**(93.0%, 98.2%)**	**2396/2421 (99.0%)**	**(98.5%, 99.3%)**
Influenza A H1	Fresh	1398	N/A[†]	N/A[†]	1398/1398 (100%)	(99.7%, 100%)
	Frozen	1242	N/A[†]	N/A[†]	1242/1242 (100%)	(99.7%, 100%)
	Total	**2640**	**N/A[†]**	**N/A[†]**	**2640/2640 (100%)**	**(99.9%, 100%)**
Influenza A H1 2009pdm[h]	Fresh	1398	29/30 (96.7%)	(83.3%, 99.4%)	1365/1368 (99.8%)	(99.4%, 99.9%)
	Frozen	1242	23/23 (100%)	(85.7%, 100%)	1213/1219 (99.5%)	(98.9%, 99.8%)
	Total	**2640**	**52/53 (98.1%)**	**(90.1%, 99.7%)**	**2578/2587 (99.7%)**	**(99.3%, 99.8%)**
Influenza A H3[i]	Fresh	1398	82/88 (93.2%)	(85.9%, 96.8%)	1306/1310 (99.7%)	(99.2%, 99.9%)
	Frozen	1242	65/69 (94.2%)	(86.0%, 97.7%)	1168/1173 (99.6%)	(99.0%, 99.8%)
	Total	**2640**	**147/157 (93.6%)**	**(88.7%, 96.5%)**	**2474/2483 (99.6%)**	**(99.3%, 99.8%)**
Influenza B[j]	Fresh	1400	7/7 (100%)	(64.6%, 100%)	1388/1393 (99.6%)	(99.2%, 99.8%)
	Frozen	1247	44/47 (93.6%)	(82.8%, 97.8%)	1191/1200 (99.2%)	(98.6%, 99.6%)
	Total	**2647**	**51/54 (94.4%)**	**(84.9%, 98.1%)**	**2579/2593 (99.5%)**	**(99.1%, 99.7%)**

Target	Storage	(n)	Positive Agreement		Negative Agreement	
			PA (%)	95% CI	NA (%)	95% CI
Mycoplasma pneumoniae[k]	Fresh	1400	8/8 (100%)	(67.6%, 100%)	1381/1392 (99.2%)	(98.6%, 99.6%)
	Frozen	1247	10/10 (100%)	(72.2%, 100%)	1228/1237 (99.3%)	(98.6%, 99.6%)
	Total	**2647**	**18/18 (100%)**	**(82.4%, 100%)**	**2609/2629 (99.2%)**	**(98.8%, 99.5%)**
Parainfluenza Virus 1[l]	Fresh	1400	4/4 (100%)	(51.0%, 100%)	1396/1396 (100%)	(99.7%, 100%)
	Frozen	1247	11/13 (84.6%)	(57.8%, 95.7%)	1234/1234 (100%)	(99.7%, 100%)
	Total	**2647**	**15/17 (88.2%)**	**(65.7%, 96.7%)**	**2630/2630 (100%)**	**(99.9%, 100%)**
Parainfluenza Virus 2[m]	Fresh	1400	2/3 (66.7%)	(20.8%, 93.9%)	1396/1397 (99.9%)	(99.6%, 100%)
	Frozen	1247	8/9 (88.9%)	(56.5%, 98.0%)	1236/1238 (99.8%)	(99.4%, 100%)
	Total	**2647**	**10/12 (83.3%)**	**(55.2%, 95.3%)**	**2632/2635 (99.9%)**	**(99.7%, 100%)**
Parainfluenza Virus 3[n]	Fresh	1400	77/79 (97.5%)	(91.2%, 99.3%)	1312/1321 (99.3%)	(98.7%, 99.6%)
	Frozen	1247	41/43 (95.3%)	(84.5%, 98.7%)	1196/1204 (99.3%)	(98.7%, 99.7%)
	Total	**2647**	**118/122 (96.7%)**	**(91.9%, 98.7%)**	**2508/2525 (99.3%)**	**(98.9%, 99.6%)**
Parainfluenza Virus 4[o]	Fresh	1400	1/1 (100%)	(20.7%, 100%)	1399/1399 (100%)	(99.7%, 100%)
	Frozen	1247	15/17 (88.2%)	(65.7%, 96.7%)	1228/1230 (99.8%)	(99.4%, 100%)
	Total	**2647**	**16/18 (88.9%)**	**(67.2%, 96.9%)**	**2627/2629 (99.9%)**	**(99.7%, 100%)**
Respiratory Syncytial Virus[p]	Fresh	1400	91/93 (97.8%)	(92.5%, 99.4%)	1293/1307 (98.9%)	(98.2%, 99.4%)
	Frozen	1247	109/111 (98.2%)	(93.7%, 99.5%)	1129/1136 (99.4%)	(98.7%, 99.7%)
	Total	**2647**	**200/204 (98.0%)**	**(95.1%, 99.2%)**	**2422/2443 (99.1%)**	**(98.7%, 99.4%)**

[†] No positive reference results recorded

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

detected and 4 were indeterminate by PCR/bi-directional sequencing.
b – *Bordetella pertussis:* Of 19 FPs, 4 were detected by PCR/bi-directional sequencing, 3 were indeterminate and 12 were not detected by PCR/bi-directional sequencing.
c – *Chlamydia pneumoniae:* The 1 FP was detected by PCR/bi-directional sequencing.
d – Coronavirus: Of 22 FNs, 5 were detected by PCR/bi-directional sequencing. 2 were not detected by PCR/bi-directional sequencing but detected by alternative NAAT. 12 were not detected by either alternative NAAT or PCR/bi-directional sequencing. 3 were not detected by PCR/bi-directional sequencing and were not tested by alternative NAAT. The 22 FPs were not detected by PCR/bi-directional sequencing.
e – Human Metapneumovirus: Of 7 FNs, 3 were detected and 4 were not detected by PCR/bi-directional sequencing. Of 17 FPs, 7 were detected while 10 were not detected by PCR/bi-directional sequencing.
f – Human Rhinovirus/Enterovirus: Of 91 FNs, 26 were detected, and 2 were indeterminate by PCR/bi-directional sequencing. 25 were not detected by PCR/bi-directional sequencing but were detected by alternative NAAT. 11 were not detected by either PCR/bi-directional sequencing or by alternative NAAT. 27 were not detected by PCR/bi-directional sequencing and had insufficient volume for alternative NAAT. Of 34 FPs, 8 were detected and 26 were not detected by PCR/bi-directional sequencing.
g – Influenza A: Of the 8 FNs, 7 were not detected by PCR/bi-directional sequencing. 1 had insufficient volume for follow-up testing. Of 25 FPs, 5 were detected by PCR/bi-directional sequencing, 19 were not detected by PCR/bi-directional sequencing, and 1 had insufficient volume for follow-up testing.
h – Influenza A H1 2009pdm: The 1 FN had insufficient volume for follow-up testing. Of 9 FPs, 4 were detected by PCR/bi-directional sequencing, and 5 were not detected by PCR/bi-directional sequencing.
i – Influenza A H3: Of 10 FNs, 7 were not detected and 1 was detected by PCR/bi-directional sequencing. 2 had insufficient volume for follow-up testing. Of 9 FPs, 3 were detected, 1 was indeterminate, and 4 were not detected by PCR/bi-directional sequencing. 1 had insufficient volume for follow-up testing.
j – Influenza B: The 3 FNs were not detected by PCR/bi-directional sequencing. Of 14 FPs, 1 was detected and 12 were not detected by PCR/bi-directional sequencing, and 1 had insufficient volume for follow-up testing.
k – *Mycoplasma pneumoniae:* Of 20 FPs, 7 were detected, 10 were not detected, and 1 was invalid by PCR/bi-directional sequencing. 2 had insufficient volume for follow-up testing.
l – Parainfluenza Virus 1: The 2 FNs were not detected by PCR/bi-directional sequencing.
m – Parainfluenza Virus 2: Of 2 FNs, 1 was detected and 1 was not detected by PCR/bi-directional sequencing. The 3 FPs were detected by PCR/bi-directional sequencing.
n – Parainfluenza Virus 3: Of 4 FNs, 2 were detected, 1 was not detected, and 1 was indeterminate, by PCR/bi-directional sequencing. Of 17 FPs, 8 were detected, 8 were not detected, and 1 was indeterminate, by PCR/bi-directional sequencing.
o – Parainfluenza Virus 4: The 2 FNs were detected by PCR/bi-directional sequencing. The 2 FPs were detected by PCR/bi-directional sequencing.
p – Respiratory Syncytial Virus: The 4 FNs were not detected by PCR/bi-directional sequencing. Of 21 FPs, 18 were not detected, 2 were detected, and 1 was indeterminate, by PCR/bi-directional sequencing.

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%).

Mixed Infections

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

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

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

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

Co-Infection Combination	Number of Specimens
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

Testing of Preselected Archived Specimens

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

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

A summary of the demographic information of the tested samples is provided in the Table which follows:

Table. Demographics of Archived Specimens

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

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

Table. Results from archived positives tested by the BioCode® RPP with easyMAG® extraction system.

Target	**(n)**	**Positive Agreement**		**Negative Agreement**	
		PA (%)	**95% CI**	**NA (%)**	**95% CI**
Adenovirus[a]	165	7/7 (100%)	(64.6%, 100%)	155/158 (98.1%)	(94.6%, 99.4%)
Bordetella pertussis[b]	165	10/10 (100%)	(72.2%, 100%)	144/155 (92.9%)	(87.7%, 96.0%)
Chlamydia pneumoniae	165	10/10 (100%)	(72.2%, 100%)	155/155 (100%)	(97.6%, 100%)
Coronavirus[c]	165	52/59 (88.1%)	(77.5%, 94.1%)	99/106 (93.4%)	(87.0%, 96.8%)
Human Metapneumovirus	165	4/4 (100%)	(51.0%, 100%)	161/161 (100%)	(97.7%, 100%)

Target	(n)	Positive Agreement		Negative Agreement	
		PA (%)	95% CI	NA (%)	95% CI
Human Rhinovirus/Enterovirus[d]	165	16/23 (69.6%)	(49.1%, 84.4%)	141/142 (99.3%)	(96.1%, 99.9%)
Influenza A	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza A H1	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza A H1 2009pdm	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza A H3	165	N/A[†]	N/A[†]	165/165 (100%)	(97.7%, 100%)
Influenza B[e]	165	2/3 (66.7%)	(20.8%, 93.9%)	162/162 (100%)	(97.7%, 100%)
Mycoplasma pneumoniae[f]	165	7/7 (100%)	(64.6%, 100%)	153/158 (96.8%)	(92.8%, 98.6%)
Parainfluenza Virus 1[g]	165	12/13 (92.3%)	(66.7%, 98.6%)	152/152 (100%)	(97.5%, 100%)
Parainfluenza Virus 2[h]	165	19/20 (95%)	(76.4%, 99.1%)	144/145 (99.3%)	(96.2%, 99.9%)
Parainfluenza Virus 3	165	1/1 (100%)	(20.7%, 100%)	164/164 (100%)	(97.7%, 100%)
Parainfluenza Virus 4[i]	165	14/15 (93.3%)	(70.2%, 98.8%)	150/150 (100%)	(97.5%, 100%)
Respiratory Syncytial Virus[j]	165	11/12 (91.7%)	(64.6%, 98.5%)	152/153 (99.3%)	(96.4%, 99.9%)

[†] *No positive reference results recorded*

a - Adenovirus: Of 3 FPs, 1 was detected by PCR/bi-directional sequencing and 2 were not detected by PCR/bi-directional sequencing.
b - *Bordetella pertussis*: Of 11 FPs, 7 were detected by PCR/bi-directional sequencing and 4 were not detected by PCR/bi-directional sequencing.
c - Coronavirus: Of 7 FNs, 4 were detected by PCR/bi-directional sequencing while 3 were not detected by PCR/bi-directional sequencing. The 7 FPs were not detected by PCR/bi-directional sequencing. All had low MFIs (<620) near the MFI cut-off on initial BioCode® RPP results.
d - Human Rhinovirus/Enterovirus: The 7 FNs were not detected by PCR/bi-directional sequencing. The 1 FP was not detected by PCR/bi-directional sequencing.
e - Influenza B: 1 FN was not detected by PCR/bi-directional sequencing.
f - *Mycoplasma pneumoniae*: Of 5 FPs, 3 were detected by PCR/bi-directional sequencing and 2 were not detected by PCR/bi-directional sequencing.
g - Parainfluenza Virus 1: 1 FN was not detected by PCR/bi-directional sequencing.
h - Parainfluenza Virus 2: 1 FN was not detected by PCR/bi-directional sequencing. 1 FP was not detected by PCR/bi-directional sequencing.
i - Parainfluenza Virus 4: 1 FN was not detected by PCR/bi-directional sequencing.
j - Respiratory Syncytial Virus: 1 FN was not detected by PCR/bi-directional sequencing. 1 FP was not detected by PCR/bi-directional sequencing.

Testing of Contrived Specimens

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

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

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

General Performance of Assay during Clinical Trials

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

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

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

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

a – 2 consecutive failures for alignment error that did not happen again after adjustment.

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

Limit of Detection

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

Table. Limit of Detection stratified by extraction system

Target	Species/Strain/Isolate	Source	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 H1 2009pdm	A(H1N1)/California/07/09	Zeptometrix 0810165CF	0.4 $TCID_{50}$/mL	20/20	0.4 $TCID_{50}$/mL	20/20
Influenza A H3	A/Wisconsin/67/05	Zeptometrix 0810252CF	4.0 $TCID_{50}$/mL	20/20	1.3 $TCID_{50}$/mL	20/20
	A/Alice	ATCC VR 776	27.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	19/20
Influenza B	Flu B/Florida/4/2006 (Yamagata)	Zeptometrix 0810255CF	0.01 $TCID_{50}$/mL	20/20	0.01 $TCID_{50}$/mL	20/20
	B/Hong Kong/S/1972 (Victoria)	ATCC VR-823	48.6 $TCID_{50}$/mL	20/20	48.6 $TCID_{50}$/mL	20/20
Respiratory Syncytial Virus	Type A	Zeptometrix 0810040ACF	0.33 $TCID_{50}$/mL	20/20	0.33 $TCID_{50}$/mL	20/20
Human Metapneumovirus	16; Type A1 IA10-2003	Zeptometrix 0810161CF	15.0 $TCID_{50}$/mL	19/20	15.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 1	C-35/Washington DC/1957	ATCC VR-94	9.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 2	Greer/Ohio/1955	ATCC VR-92	1.8 $TCID_{50}$/mL	20/20	5.4 $TCID_{50}$/mL	20/20
Parainfluenza Virus 3	N/A	Zeptometrix 0810016CF	15.0 $TCID_{50}$/mL	20/20	15.0 $TCID_{50}$/mL	20/20
Parainfluenza Virus 4	Type 4a	Zeptometrix 0810060CF	9.0 $TCID_{50}$/mL	20/20	9.0 $TCID_{50}$/mL	20/20
Adenovirus	Species B Serotype 7A	Zeptometrix 0810021CF	1.2 $TCID_{50}$/mL	20/20	1.2 $TCID_{50}$/mL	20/20
Adenovirus	Species C Serotype 2	ATCC VR-846	6.0 $TCID_{50}$/mL	20/20	18.0 $TCID_{50}$/mL	20/20
Adenovirus	Species E Serotype 4	Zeptometrix 0810070CF	0.04 $TCID_{50}$/mL	20/20	0.04 $TCID_{50}$/mL	19/20

Target	Species/Strain/Isolate	Source	easyMAG®		MagNA Pure 96	
			Concentration	Detected (n of 20)	Concentration	Detected (n of 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]	$5.0x10^4$ copies/mL	19/20	$5.0x10^4$ 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 (copies/mL) with Applied BioCode validated SYBR assay using an IVT RNA standard.

Analytical Reactivity (Inclusivity)

A study was performed to verify Analytical Reactivity/Inclusivity of the BioCode® Respiratory Pathogen Panel (RPP). Different strains, serotypes and genotypes were selected that represent various temporal, geographic, and genetic diversity for each analyte. This study tested a panel of titered stocks for relevant targets diluted in simulated NPS in UTM matrix. Assay reactivity for less common strains or serotypes that could not be tested due to unavailability was predicted using *in silico* analysis.

Table. Influenza A isolates tested during inclusivity.

Organism/ Type[a]	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Influenza A H1N1	Solomon Island/03/06	Zeptometrix	0810036CFN	45 $TCID_{50}$/mL	3x
	Singapore/63/04	Zeptometrix	0810246CF	45 $TCID_{50}$/mL	3x
	PR/8/34	Zeptometrix	0810245CF	45 $TCID_{50}$/mL	3x
	A/Brisbane/59/2007	Zeptometrix	0810244CF	45 $TCID_{50}$/mL	3x
	A/Taiwan/42/06	Zeptometrix	0810247CF	45 $TCID_{50}$/mL	3x
	A/New Jersey/8/76[b]	ATCC	VR-897	$7.5x10^3$ $CEID_{50}$/mL	500x[b]
	A/Denver/1/1957	VIRAPUR	N/A	45 $TCID_{50}$/mL	3x
	A/FM/1/47	ATCC	VR-97	45 $CEID_{50}$/mL	3x
	A/Weiss/43[c]	ATCC	VR-96	$1.5x10^4$ $CEID_{50}$/mL	1000x[c]
	A/Beijing/262/95[d]	BEI	NR-12277	450 $CEID_{50}$/mL	30x[d]
	A/Mal/302/54	ATCC	VR-98	45 $CEID_{50}$/mL	3x
Influenza A H1N2	Recombinant; Kilbourne F64, A/NWS/1934 (HA) x A/Rockefeller Institute/ 5/1957 (NA)[e]	BEI	NR-3682	135 $CEID_{50}$/mL	5x[e]
Influenza A H1N1 pdm09	NY/01/09	Zeptometrix	0810248CF	1.2 $TCID_{50}$/mL	3x
	NY/02/09	Zeptometrix	0810109CFN	1.2 $TCID_{50}$/mL	3x
	NY/03/09	Zeptometrix	0810249CF	1.2 $TCID_{50}$/mL	3x
	A/Houston/3H/2009 (H1N1)pdm09[f]	BEI	NR-20340	1.2 $TCID_{50}$/mL	3x
	Influenza A H1N1pdm (Canada/6294/09)	Zeptometrix	0810109CFJ	1.2 $TCID_{50}$/mL	3x
	Influenza A H1N1pdm (Mexico/4108/09)	Zeptometrix	0810166CF	1.2 $TCID_{50}$/mL	3x
	California/04/09, cell isolate[g]	BEI	NR-13658	40 $TCID_{50}$/mL	100x[g]
	A/Christ Church/16/2010[h]	CDC	N/A	400 EID_{50}/mL	1000x[h]
	A/Brisbane/02/2018	CDC	N/A	40 EID_{50}/mL	100x[i]

Organism/ Type[a]	Strain/Location/Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected
Influenza A H3N2	A/Wisconsin/15/2009[j]	BEI	NR-42007	12 $CEID_{50}$/mL	3x
	A/Texas/50/12	Zeptometrix	0810238CF	12 $TCID_{50}$/mL	3x
	A/Brisbane/10/2007	Zeptometrix	0810138CF	12 $TCID_{50}$/mL	3x
	A/Port Chalmers/1/73	ATCC	VR-810	200 $CEID_{50}$/mL	50x[k]
	A/Victoria/3/1975	VIRAPUR	NA	12 $TCID_{50}$/mL	3x
	A/Victoria/361/2011[l]	BEI	NR-44022	12 $CEID_{50}$/mL	3x
	A/Victoria/3/75	ATCC	VR-822	12 $CEID_{50}$/mL	3x
	A/Uruguay/716/07[m]	BEI	NR-42003	40 $TCID_{50}$/mL	10x
	A/HK/H090-756-V1(0)/2009[n]	BEI	NR-44344	12 $TCID_{50}$/mL	3x
	A/Hong Kong/8/68	Zeptometrix	0810250CF	12 $TCID_{50}$/mL	3x
	A/Switzerland/9715293/13	VIRAPUR	NA	12 $TCID_{50}$/mL	3x
	A/Aichi/2/68	ATCC	VR-547	12 $TCID_{50}$/mL	3x
	MRC-2	ATCC	VR-777	12 $TCID_{50}$/mL	3x
	A/Perth/16/2009	CDC	N/A	40 EID_{50}/mL	10x
	A/Kansas/14/2017[o]	CDC	N/A	8000 EID_{50}/mL	2000x[o]

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

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

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

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

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

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

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

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

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

j – Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Wisconsin/15/2009 (H3N2), NR-42007.
k - *In silico* analysis revealed a few non-critical mismatches in the FluA/H3 subtyping HA assay probe binding region that could contribute to the observed lower reactivity. However, titering inconsistency from the vendor rather than reduced reactivity due to assay design is suspected.
l - Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Victoria/361/2011 (H3N2), NR-44022
m - Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/Uruguay/716/07 (H3N2), NR-42003
n - Virus obtained through BEI Resources, NIAID, NIH: Influenza A, A/HK/H090-756-V1(0)/2009 (H3N2), NR-44344
o – Virus obtained through the CDC Influenza Division. This strain was detected by BioCode® RPP as Flu A (no subtype) at 50xLoD. *In silico* analysis did not reveal any mismatch in the Flu A matrix assay primers and probe binding regions but showed 3 mismatches in the Flu A/H3 subtyping HA assay primers binding regions that could predict reduced analytical reactivity, 1 mismatch at the 9th position from the 3' end of the reverse primer (mismatch #1), 1 mismatch at the 18th position from the 3' end of the reverse primer (mismatch #2), and 1 mismatch at the 20th position from the 3' end of the reverse primer (mismatch #3). Wet testing data suggested that mismatch #1 is likely the root cause for the observed significant reduction in analytical reactivity for this strain. For patient samples that contain a Flu A/H3 strain that harbors mismatch #1 at lower concentrations, the BioCode® RPP will likely report a Flu A (no subtype detected) result. Although estimated prevalence of a sequence variant based solely on *in silico* analysis may not accurately reflect the actual prevalence of the sequence variant in circulation during an influenza season, based on an *in silico* analysis, of all the Flu A/H3 strains isolated in 2019 with published HA sequences, 73.8% of the strains harbor this particular sequence mismatch.

Table. Influenza B isolates tested during the inclusivity study

Organism/Lineage	Location/Strain/ Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[a]
Influenza B (Victoria[b])	B/Malaysia/2506/2004[d]	BEI	NR-9723	1.458 $TCID_{50}$/mL	0.03x
	B/Malaysia/2506/2004	Zeptometrix	0810258CF	1.458 $TCID_{50}$/mL	0.03x
	B/Ohio/01/2005[e]	BEI	NR-41801	14.58 $CEID_{50}$/mL	0.3x
	B/Brisbane/33/2008[f]	BEI	NR-42006	14.58 $CEID_{50}$/mL	0.3x
	B/Nevada/03/2011[g]	BEI	NR-44023	1.458 $CEID_{50}$/mL	0.03x
	B/Michigan/09/2011	CDC	N/A	14.58 EID_{50}/mL	0.3x
	B/Colorado/06/2017	CDC	N/A	14.58 EID_{50}/mL	0.3x
Influenza B (Yamagata)	B/Texas/06/2011[h]	BEI	NR-44024	50 $TCID_{50}$/mL	5000x[h]
	B/Sydney/507/2006[i]	BEI	NR-36526	8.0 $TCID_{50}$/mL	800x[i]
	B/Wisconsin/1/10	Zeptometrix	0810241CF	0.03 $TCID_{50}$/mL	3x
	B/Massachusetts/2/12	Zeptometrix	0810239CF	0.03 $TCID_{50}$/mL	3x
	B/Christchurch/33/2004[j]	BEI	NR-36536	0.03 $TCID_{50}$/mL	3x
	B/New Hampshire/01/2016[k]	CDC	N/A	10 EID_{50}/mL	1000x[k]
	B/Phuket/3073/2013[l]	CDC	N/A	5 EID_{50}/mL	500x[l]
Influenza B (unknown lineage[c])	B/Lee/1940	Zeptometrix	0810257CF	0.03 $TCID_{50}$/mL	3x
	B/Taiwan/2/1962	ATCC	VR-295	5.0 $CEID_{50}$/mL	500x[m]

Organism/Lineage	Location/Strain/ Year	Vendor	Catalog #	Concentration Detected	Multiple of LoD Detected[a]
	B/Allen/45	ATCC	VR-102	0.05 $CEID_{50}$/mL	5x
	B/Brigit	ATCC	VR-786	0.03 $TCID_{50}$/mL	3x

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

Table. Respiratory Syncytial Virus isolates tested during the inclusivity study

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

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

Table. Human Metapneumovirus isolates tested during the inclusivity study

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

a - If either hMPV1 or hMPV2 assay has MFI above the cutoff, the software will report as Detected for Human Metapneumovirus
b - Virus obtained through BEI Resources, NIAID, NIH: Human Metapneumovirus, TN/91-316, NR-22232

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

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

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

Table. Adenovirus isolates tested during the inclusivity study

Species[b]	Serotype	Strain/Location/Year	Vendor	Catalog #	Concentration Detected[c]	Multiple of LoD Detected
Adenovirus A[a]	31	Unknown	Zeptometrix	0810073CF	18 $TCID_{50}$/mL	3x
	12	Huie/Massachusetts	ATCC	VR-863	18 $TCID_{50}$/mL	3x
	18	Washington DC/1954	ATCC	VR-19	18 $TCID_{50}$/mL	3x
Adenovirus B	3	GB/Maryland/1953	ATCC	VR-3	3.6 $TCID_{50}$/mL	3x
	14	Unknown	Zeptometrix	0810108CF	3.6 $TCID_{50}$/mL	3x
	16	CH.79/Saudi Arabia/1955	ATCC	VR-17	3.6 $TCID_{50}$/mL	3x
	35	Holden	ATCC	VR-718	3.6 $TCID_{50}$/mL	3x
Adenovirus C	1	Unknown	Zeptometrix	0810050CF	18 $TCID_{50}$/mL	3x
	5	Unknown	Zeptometrix	0810020CF	18 $TCID_{50}$/mL	3x
	6	Tonsil 99/Washington DC	ATCC	VR-6	18 $TCID_{50}$/mL	3x
Adenovirus D[a]	8	Unknown	Zeptometrix	0810069CF	18 $TCID_{50}$/mL	3x
	17	CH. 22/Saudi Arabia/1955	ATCC	VR-1836	18 $TCID_{50}$/mL	3x
	20	Unknown	Zeptometrix	0810115CF	18 $TCID_{50}$/mL	3x
	26	Unknown	Zeptometrix	0810117CF	18 $TCID_{50}$/mL	3x
	37	Unknown	Zeptometrix	0810119CF	18 $TCID_{50}$/mL	3x
Adenovirus F[a]	40	Unknown	Zeptometrix	0810084CF	18 $TCID_{50}$/mL	3x
	41	Tak/73-3544/ Netherlands/1973	ATCC	VR-930	18 $TCID_{50}$/mL	3x

a – Adenovirus C LoD used as LoD was not determined for Species A, D or F.
b – *In silico* analysis predicts detection of Adenovirus species E serotypes (Adenovirus 4 strain was tested as a part of LoD studies as well.
c – If either ADV1 or ADV2 assay has MFI above the cutoff the software will report as Detected for Adenovirus.

Table. Coronavirus isolates tested during the inclusivity study

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

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

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

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

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

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

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

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

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

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

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)
- A/Ohio/09/2015 (H1N1v)
- A/Ohio/35/2017(H1N2v)
- A/Ohio/13/2017(H3N2v)
- A/gryfalcon/Washington/41088-6/2014 (H5N8)
- A/Northern Pintail/Washington/40964/2014(H5N2)
- A/Anhui/1/2013(H7N9)
- A/Bangladesh/0994/2011(H9N2)

Consistent with the *in silico* predictions of analytical reactivity to zoonotic influenza A viruses, each non-seasonal influenza A strain tested (H2N2, H5N1, H5N2, H9N2, H7N9, and H5N8 was detected as Influenza A (no subtype) at various concentrations tested (i.e., detected by the BioCode® RPP Influenza A matrix assay only). H1 (H1N1v, H1N1v) and H3 (H3N2v) strains, were detected also by respective subtypes at higher concentration in addition to detection of influenza A matrix. However, at lower concentrations, these strains may be detected as influenza A (no subtype), detected only by influenza A matrix assay of BioCode® RPP.

Analytical Specificity (Cross-Reactivity and Exclusivity)

A study was performed to verify that the BioCode® Respiratory Pathogen Panel (RPP) does not detect DNA or RNA from off-panel organisms and viruses commonly found in respiratory specimens or from organisms and viruses that can cause similar clinical symptoms. In addition, on-panel targets were tested at high concentrations to ensure there is no cross-reactivity with other on-panel targets. Organisms and viruses that were not available for wet testing were analyzed *in silico* comparing the whole genome against all primers to assess potential for cross reactivity. Analysis was conducted using BLASTn and Primer-BLAST programs. Microorganisms were 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. Stocks were combined with simulated NPS in UTM matrix at the time of extraction. Each organism was extracted in triplicate on the easyMAG® and assayed in singlet with the RPP on the BioCode® MDx-3000 system according to the instructions for use. For each concentration tested, the number of replicates that gave valid results per the Interpretation Algorithm was determined. If any replicates were detected, testing was repeated from 5 additional extractions assayed in singlet. If detected after repeat with 5 additional replicates, serial dilutions were performed to determine the lower limit.

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

Organism	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/N)
Acinetobacter baumannii	Zeptometrix	801597	9.67 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
Proteus vulgaris	Zeptometrix	801898	4.13 x 10^7 CFU/mL	N
Pseudomonas aeruginosa	ATCC	39324	2.11 x 10^6 CFU/mL	N

Organism	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/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

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

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

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

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

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

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

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

Pathogen	Vendor	Catalog #	Titer tested	Cross-reactivity (Y/N)
Influenza A H1N1/New Caledonia/20/99	Zeptometrix	0810036CF	1.15 x 10^5 $TCID_{50}$/mL	N
Influenza A H1N1 /NWS/33	ATCC	VR-219	7.40 x 10^5 $TCID_{50}$/mL	N
Influenza A H1N1 pdm09/California/07/09	Zeptometrix	0810165CF	1.31 x 10^5 $TCID_{50}$/mL	N
Influenza A H3N2 /Wisconsin/67/05a	Zeptometrix	0810252CF	1.08 x 10^5 $TCID_{50}$/mL	N
Influenza A H3N2/Alice	ATCC	VR-776	1.43 x 10^6 $TCID_{50}$/mL	N
Influenza B/Florida/4/2006 (Yamagata)	Zeptometrix	0810255CF	1.08 x 10^5 $TCID_{50}$/mL	N
Influenza B/Hong Kong/S/1972 (Victoria)	ATCC	VR-823	8.57 x 10^5 $TCID_{50}$/mL	N
Respiratory Syncytial Virus (Type A)	Zeptometrix	0810040ACF	4.57 x 10^5 $TCID_{50}$/mL	N
Human Metapneumovirus 16 (Type A1)	Zeptometrix	0810161CF	8.51 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 1	ATCC	VR-94	1.60 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 2	ATCC	VR-92	1.35 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 3	Zeptometrix	0810016CF	3.39 x 10^5 $TCID_{50}$/mL	N
Parainfluenza Virus 4a	Zeptometrix	0810060CF	1.13 x 10^5 $TCID_{50}$/mL	N
Adenovirus Species B Serotype 7A	Zeptometrix	0810021CF	5.83 x 10^5 $TCID_{50}$/mL	N
Adenovirus Species C Serotype 2	ATCC	AV-846	2.81 x 10^5 $TCID_{50}$/mL	N
Adenovirus Species E Serotype 4	Zeptometrix	0810070CF	1.08 x 10^5 $TCID_{50}$/mL	N
Coronavirus 229E	Zeptometrix	0810229CF	1.09 x 10^5 $TCID_{50}$/mL	N
Coronavirus HKU1	N/A	Clinical sample[a]	1.92 x 10^5 Copies/mL	N
Coronavirus NL63	Zeptometrix	0810228CF	1.08 x 10^5 $TCID_{50}$/mL	N
Coronavirus OC43	Zeptometrix	0810024CF	1.08 x 10^5 $TCID_{50}$/mL	N
Human Rhinovirus Type A1	Zeptometrix	0810012CF	1.05 x 10^5 $TCID_{50}$/mL	N
Enterovirus D68	Zeptometrix	0810300CF	1.08 x 10^5 $TCID_{50}$/mL	N
Bordetella pertussis	Zeptometrix	801459	3.86 x 10^7 CFU/mL	N
Mycoplasma pneumoniae	Zeptometrix	801579	1.06 x 10^6 CCU/mL	N
Chlamydia pneumoniae (AR-39)	ATCC	53592	1.24 x 10^6 CFU/mL	N
Chlamydia pneumoniae (CWL-029)	ATCC	VR-1310	1.00 x 10^6 CFU/mL	N

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

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

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

Cross-Contamination and Carryover

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

Reproducibility

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

Table. Reproducibility panel

Medium (3x LoD)	Medium (3x LoD)	Low (1.5x LoD)	Low (1.5x LoD)	Sample Name
Human Rhinovirus	Parainfluenza Virus 2	Human Metapneumovirus	*Bordetella pertussis*	RP1
Human Metapneumovirus	*Bordetella pertussis*	Human Rhinovirus	Parainfluenza Virus 2	RP2
Influenza B	Coronavirus NL63	*Chlamydia pneumoniae*	Parainfluenza Virus 3	RP3
Chlamydia pneumoniae	Parainfluenza Virus 3	Influenza B	Coronavirus NL63	RP4
Influenza A H3N2	*Mycoplasma pneumoniae*	Respiratory Syncytial Virus	Adenovirus C	RP5
Respiratory Syncytial Virus	Adenovirus C	Influenza A H3N2	*Mycoplasma pneumoniae*	RP6
Simulated Negative matrix				RP7

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

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

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result					
			NucliSENS® easyMAG®			MagNA Pure 96		All Sites
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	(95% CI)
Viruses								
Adenovirus	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**149/150** 99.30%	**299/300** 99.70%	**150/150** 100%	**150/150** 100%	**449/450** **99.80%** (98.8%-100%)
Coronavirus NL63	**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
			Site 1	Site 3	Sub-Total	Site 2	Sub-Total	(95% CI)
Viruses								
Influenza A/H3	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**29/30**[a] 96.70%	**30/30** 100%	**59/60** 98.30%	**30/30** 100%	**30/30** 100%	**89/90** **98.90%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**149/150**[b] 99.30%	**299/300** 99.70%	**150/150** 100%	**150/150** 100%	**449/450** **99.80%** (98.8%-100%)
Influenza A/H1pdm09	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Influenza A/H1	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Influenza B	**3× LoD**	**Detected**	**30/30** 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** 96.70%	**30/30** 100%	**59/60** 98.30%	**30/30** 100%	**30/30** 100%	**89/90** **98.90%** (94.0%-99.8%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Parainfluenza Virus 1	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)
Parainfluenza Virus 2	**3× LoD**	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	1.5× LoD	**Detected**	**30/30** 100%	**30/30** 100%	**60/60** 100%	**30/30** 100%	**30/30** 100%	**90/90** **100%** (95.9%-100%)
	None (no analyte)	**Not Detected**	**150/150** 100%	**150/150** 100%	**300/300** 100%	**150/150** 100%	**150/150** 100%	**450/450** **100%** (99.2%-100%)
Parainfluenza Virus 4	**None** (no analyte)	**Not Detected**	**210/210** 100%	**210/210** 100%	**420/420** 100%	**210/210** 100%	**210/210** 100%	**630/630** **100%** (99.4%-100%)

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

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

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

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

Interference

A study was performed to demonstrate the accuracy of the BioCode® Respiratory Pathogen Panel on the BioCode® MDx-3000 in the presence of potentially inhibiting substances or microorganisms. Each member of the interfering substance panel was added to simulated NPS (sNPS) in UTM matrix containing representative members of the BioCode® RPP at 3X LoD and a negative sample comprised of only sNPS in UTM matrix. Each sample was tested with and without potentially interfering substances 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 tested at lower concentrations.

Table. Contrived samples (3x LoD in sNPS)

Sample Name	Pathogen	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

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

Table. Evaluation for microbial interferents on BioCode® RPP

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

Table. Non-microbial interfering substances tested for BioCode® RPP assay

Substance Interferent	Brand/Source	Concentration	Interference Yes (Y) or No (N)
Genomic DNA	Promega	10 ng/µl	N
Mucin (MagNA Pure 96)	Sigma	0.6% W/V	N
Mucin (easyMAG®)[a]	Sigma	0.5% W/V	N
Human Blood	Poplar Health	1% V/V	N
Zanamivir	APExBIO	550 ng/mL	N
Oseltamivir	APExBIO	142 ng/mL	N
Nasal spray	Equate	1% V/V	N
Nasal decongestant spray	Bayer	1% V/V	N

Substance Interferent	Brand/Source	Concentration	Interference Yes (Y) or No (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

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

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

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

a - 2/3 replicates detected

None of the substances were shown to interfere with BioCode® RPP at the concentrations tested. However, it was observed that mucin at higher concentration (0.6%) could lead to loss of signal for some targets (loss of analyte detection) when extracted with the easyMAG®. The effect of mucin was dependent on the concentration in the sample tested. FluMist was evaluated to be reactive as predicted with BioCode® RPP assay, therefore recent administration or contamination of specimens by flu vaccine prior to NPS collection could lead to false detection by BioCode® RPP.

Competitive Inhibition

A study was performed to evaluate the potential for inhibition in samples with mixed infections. Targets were spiked into simulated NPS in UTM matrix with one target at high concentration (≥10^6 CFU/mL for bacteria and ≥10^5 units/mL for viruses) and two targets at low concentration (≤3x LoD). Common co-infections were determined by reviewing results of previous Respiratory Panel clinical trials from 510k summaries, publications/posters and internal clinical sample testing. Each sample was extracted in triplicate on the easyMAG® and each extraction tested in singlet with the RPP on the BioCode® MDx-3000 system.

Table. Competitive inhibition testing results

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

Panel Designation	Viral/Bacteria Strain	Source	Level	Titer Tested	Result (n of 3 Detected)
Competitive Inhibition Sample 7	Influenza A H1N1 pdm California/07/09	Zeptometrix 0810165CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
					3/3
	Parainfluenza Virus 3	Zeptometrix 0810016CF	Low	45 $TCID_{50}$/mL	3/3
	Human Rhinovirus type A	Zeptometrix 0810012CFN	Low	3.6 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 8	Parainfluenza Virus 3	Zeptometrix 0810016CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Human Rhinovirus type A	Zeptometrix 0810012CFN	Low	3.6 $TCID_{50}$/mL	3/3
	Influenza A H1N1 pdm California/07/09	Zeptometrix 0810165CF	Low	1.2 $TCID_{50}$/mL	3/3
					3/3
Competitive Inhibition Sample 9	Human Rhinovirus type A	Zeptometrix 0810012CFN	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza A H1N1 pdm California/07/09	Zeptometrix 0810165CF	Low	1.2 $TCID_{50}$/mL	3/3
					3/3
	Parainfluenza Virus 3	Zeptometrix 0810016CF	Low	45 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 10	*Mycoplasma pneumoniae*	Zeptometrix 801579	High	$1x10^6$ CCU/mL	3/3
	Coronavirus NL63	Zeptometrix 0810228CF	Low	1.2 $TCID_{50}$/mL	3/3
	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	Low	0.04 $TCID_{50}$/mL	3/3
Competitive Inhibition Sample 11	Coronavirus NL63	Zeptometrix 0810228CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	Low	0.04 $TCID_{50}$/mL	3/3
	Mycoplasma pneumoniae	Zeptometrix 801579	Low	45 CCU/mL	3/3
Competitive Inhibition Sample 12	Influenza B/Florida/4/2006	Zeptometrix 0810255CF	High	$1x10^5$ $TCID_{50}$/mL	3/3
	Mycoplasma pneumoniae	Zeptometrix 801579	Low	45 CCU/mL	3/3
	Coronavirus NL63	Zeptometrix 0810228CF	Low	1.2 $TCID_{50}$/mL	3/3

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

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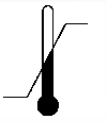
TABLE OF SYMBOLS

The following symbols are used on the BioCode® Respiratory Pathogen Panel kit components and/or in this package insert.

LOT	Batch code		Date of Manufacture		Temperature limitations
REF	Catalog number		Contains sufficient for <n> tests		Consult instructions for use
	Use by YYYY-MM-DD		Do Not Reuse		Manufacturer
IVD	*In vitro* diagnostic device		For Prescription Use Only		European Union Compliance Label
	General Cautions and Warnings				

Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests