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NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Package Insert

Rx Only

IVD For *In Vitro* Diagnostic Use.

For use under an Emergency Use Authorization (EUA) only.

MLD-056-KPI-001 Rev A
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NxTAG Respiratory Pathogen Panel + SARS-CoV-2 (IVD)

Σ 96 TESTS

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Symbols Glossary

You will encounter these symbols throughout this manual. They represent warnings, conditions, identifications, instructions, and regulatory agencies.

Symbol	Meaning	Symbol	Meaning
5.4.4* 	Caution. Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.	5.1.4* 	Use-by date. Indicates the date after which the medical device is not to be used.
5.1.5* 	Batch Code. Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.1* 	Manufacturer. Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
5.5.5* 	Contains Sufficient for <n> Tests. Indicates the total number of IVD tests that can be performed with the IVD.	5.3.7* 	Temperature Limit. Indicates the temperature limits to which the medical device can be safely exposed.
5.4.3* 	Consult instructions for use. Indicates the need for the user to consult the instructions for use.	5.1.6* 	Catalog(ue) Number. Indicates the manufacturer's catalogue number so that the medical device can be identified.
5.5.1* 	*In vitro* diagnostic medical device. Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.	5.2.8* 	Do not use if package is damaged. Indicates a medical device that should not be used if the package has been damaged or opened.
5.3.4* 	Keep dry. Indicates a medical device that needs to be protected from moisture.	† 	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner (U.S. Only)

* ANSI/AAMI/ISO 15223-1:2016, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

† 21 CFR 809 (FDA Code of Federal Regulations).

Luminex Technical Support

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Additional information is available on the website. Search on the desired topic, navigate through menus. Also, review the website's FAQ section. Enter *http://www.luminexcorp.com* in your browser's address field.

This manual can be updated periodically. To ensure that you have a current version, contact Technical Support.

Intended Use

NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) for use on Luminex® MAGPIX® instrument is a multiplexed nucleic acid RT-PCR test intended for the simultaneous qualitative detection and differentiation of nucleic acid from multiple viral and bacterial respiratory organisms, including nucleic acid from SARS-CoV-2, in nasopharyngeal swabs from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and the targeted respiratory viral and bacterial organisms can be similar. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.

NxTAG RPP + SARS-CoV-2 is intended for use in the simultaneous detection and differentiation of nucleic acids from SARS-CoV-2 and the following organism types and subtypes identified using NxTAG RPP + SARS-CoV-2: Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Bocavirus, *Chlamydophila pneumoniae*, and/or *Mycoplasma pneumoniae*.

SARS-CoV-2 RNA and nucleic acids from the other respiratory viral and bacterial organisms identified by this test are generally detectable in a nasopharyngeal swab specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and 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. Positive results are indicative of the presence of the identified organism, but do not rule out co-infection with other pathogens. The agent(s) detected by the NxTAG RPP + SARS-CoV-2 may not be the definite cause of disease.

Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results in the setting of a respiratory illness may be due to infection with pathogens not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative SARS-CoV-2 results must be combined with clinical observations, patient history, and epidemiological information. Negative results for other organisms reported by the test may require additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence and radiography) when evaluating a patient with possible respiratory tract infection.

The NxTAG RPP + SARS-CoV-2 is intended for use by qualified laboratory personnel specifically instructed and trained in the operation of the Luminex MAGPIX instrument and *in vitro* diagnostic procedures. The NxTAG RPP + SARS-CoV-2 is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

Respiratory Pathogens

Respiratory viruses are a leading cause of morbidity, hospitalization, and mortality worldwide. They cause acute local and systemic illnesses that range in severity, and have the potential to cause severe disease especially in the young and elderly. Respiratory viruses are highly prevalent and are the most common cause of acute illness and physician visits in the U.S. (Tsukagoshi et al. 2013). The frequency of respiratory viral infections is highest in children under 4 years of age. School children get infected, on average, with 5 to 8 respiratory viruses per year, and adults average 2 to 4 respiratory viruses per year (Monto 1994; Turner 1998; Khabbaz et al. 2010). Bacteria that cause respiratory infections represent approximately 10% of all upper respiratory tract infections. However, antibiotics are often prescribed for respiratory infections despite the viral etiology in 90% of cases (Berry et al. 2015). Clarity on the causative pathogen in respiratory illness aids patient diagnosis and treatment management and can help to reduce over prescribing of antibiotics.

Influenza Type A and B

Influenza Type A and B viruses cause annual epidemics that affect 5% to 20% of the U.S. population during the winter months (Biggerstaff et al. 2014). They are members of the *Orthomyxoviridae* family, and are small enveloped particles with an antisense RNA genome (Cheng et al. 2012). Influenza A and B strains undergo genetic variation, creating different strains that all or part of the human population may be vulnerable. Influenza A viruses have two subtypes that are particularly important for human infections: H3N2 and H1N1. In 2009, a novel Influenza A H1N1 strain (2009 H1N1) was identified. Influenza A is usually a more severe infection than type B, and H3N2 strains have higher mortality. Another subtype of influenza viruses has had some relevance to humans: avian H5N1. This strain, previously infecting only birds, has been infecting humans through contact with birds resulting in a number of outbreaks in Southeast Asia and the Middle East (Biggerstaff et al. 2014). Influenza viruses are generally transmitted by droplets with an incubation period of 1 to 4 days (La Rosa et al. 2013; Lessler et al. 2009). In North America, infection tends to occur in the winter months (Azziz Baumgartner et al. 2012).

Respiratory Syncytial Virus (RSV)

Respiratory Syncytial Virus (RSV) is a member of the Paramyxoviridae family, and is a medium sized, enveloped virus with an antisense RNA genome (Chidgey and Broadley 2005). There are two subtypes of RSV, type A and type B. RSV is identified using the RNA polymerase L gene. Illness caused by type A RSV may be more clinically severe than illness caused by type B. Transmission is via contact and through inhalation of droplets, with an incubation period of 3 to 7 days (La Rosa et al. 2013; Lessler et al. 2009). The incidence of RSV infections is seasonal, with outbreaks from November to April, peaking in December, January, and February (Chidgey and Broadley 2005; Simoes 2008). Globally, RSV is responsible for one third of the deadly childhood pneumonia cases (Meng et al. 2014).

Human Metapneumovirus (hMPV)

Human Metapneumovirus (hMPV) is a recently discovered virus found worldwide, and is the cause of significant upper and lower respiratory infections in all age groups,accounting for 7% to 19% of all infections in children.. hMPV is a member of the Paramyxoviridae family, which also includes RSV and parainfluenza. Viruses in the Paramyxoviridae family are enveloped particles containing an antisense RNA genome. hMPV is identified in this assay using the phosphoprotein (P) gene. Two major lineages of hMPV exist, A and B (Berry, et al. 2015). Transmission is likely to occur by direct or close contact with contaminated secretions; nosocomial infections have also been reported. Limited studies suggest an incubation period of 4 to 6 days (Haas et al. 2013; Lessler et al. 2009). hMPV outbreaks are seasonal, and parallel RSV outbreaks, with peak incidence from December to April (Mullins et al., 2004; Williams et al. 2004; Kroll and Weinberg 2011; Berry et al. 2015).

Rhinovirus

Rhinoviruses are extremely frequent causes of respiratory infections, causing over half of infections (Anzueto and Niederman 2003; Makela et al. 1998; Greenberg 2011). Rhinoviruses are members of the Picornaviridae family, which also includes enteroviruses. Members of the Picornaviridae family are small, non-enveloped particles containing an RNA genome. Variations of the capsid protein encasing the genome give rise to greater than 100 serotypes of rhinovirus (Greenberg 2011; Pitkaranta and Hayden 1998). The 5′ untranslated region is used for detection of rhinoviruses in this assay. The incidence of rhinoviruses is seasonal, with peaks in the fall and early spring (Anzueto and Niederman 2003; Greenberg 2011). Rhinoviruses can be the causative organism in up to 80% of colds in September and October (Arruda et al. 1997). In general transmission is via large droplets with an incubation period of 2 to 4 days (La Rosa et al. 2013; Lessler et al. 2009).

Enterovirus

Enteroviruses are very common causes of infections that have a variety of clinical manifestations, from minor febrile illness to severe, potentially fatal conditions such as aseptic meningitis, paralysis, myocarditis, and neonatal enteroviral sepsis (Khetsuriani et al. 2006). Enteroviruses are members of the Picornaviridae family, which also includes rhinoviruses. Members of the Picornaviridae family are small, non-enveloped particles containing an RNA genome. The 5′ untranslated region is used for detection of enteroviruses in this assay. Many different serotypes of enterovirus exist including 28 serotypes of echovirus, 23 serotypes of coxsackievirus A, 6 serotypes of coxsackievirus B, 4 serotypes of enteroviruses 68 to 71, and 3 serotypes of poliovirus (Khetsuriani et al. 2006; Stalkup and Chilukuri 2002; Yarush and Steele 2000). The peak incidence of enterovirus infection occurs in the mid-summer to early fall with transmission occurring via fecal-oral mode (Khetsuriani et al. 2006; Stalkup and Chilukuri 2002; Yarush and Steele 2000; La Rosa et al. 2013). Incubation time is 3 to 7 days (Flor de Lima et al. 2013).

Parainfluenza (PIV)

Parainfluenza viruses (PIV) are a common cause of upper and lower respiratory infections and croup, especially in children (Frost et al. 2014; Liu et al. 2013). In all croup cases from which viruses can be isolated, 60% of the isolates are parainfluenza viruses. Parainfluenza viruses are also the second leading contributor to pediatric hospitalization for respiratory disease (Wright 2010). Parainfluenza viruses are members of the Paramyxoviridae family, which also includes RSV. Viruses in the Paramyxoviridae family are enveloped particles with antisense, single-stranded RNA genomes. Four serotypes of PIV can cause disease in humans: parainfluenza 1 to 4 (PIV1, PIV2, PIV3, and PIV4). PIV1 is identified using the hemagglutinin neuraminidase (HN) gene and PIV4 uses the phosphoprotein (P) gene. Both PIV2 and PIV3 are identified using the nucleocapsid protein (NP) gene. PIV1 and PIV2 are most prevalent in the fall, with biennial outbreaks for PIV1. PIV3 can be found all year, but is most prevalent in the spring and early summer (Fry et al. 2006; Henrickson et al. 2003). Limited studies show a varied PIV4 prevalence with some reporting year round infection with biennial peaks in odd-years, others with winter to spring infection, and yet others with no pattern, making PIV4 seasonality difficult to determine (Frost et al. 2014; Liu et al. 2013; Abiko et al. 2013; Fairchok et al. 2011; Vachon et al. 2006). Transmission is via aerosolization of large droplets with an incubation period of 2 to 6 days (Hendrickson et al. 2003; Lessler et al. 2009).

Coronavirus

There is currently an outbreak of respiratory disease caused by a novel coronavirus that was first detected in Wuhan City, Hubei Province, China, which has now been designated a pandemic by the World Health Organization (WHO) and which has been detected internationally, including cases in the United States. The virus has been named "SARS-CoV-2" and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption. The potential public health threat posed by COVID-19 is high, both globally and to the United States. To respond effectively to the COVID-19 outbreak, rapid detection of cases and contacts, appropriate clinical management and infection control, and implementation of community mitigation efforts are critical. On February 4, 2020, the Secretary of Health and Human Services (HHS) determined that there is a public health emergency and that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of the novel coronavirus (2019-nCoV). Rapid detection of COVID-19 cases in the United States requires wide availability of diagnostic testing to control the emergence of this rapidly spreading, severe illness. [FDA - Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency, March 16, 2020].

Coronaviruses other than SARS-CoV-2 are the second most common cause of colds, after rhinoviruses. During peak coronavirus season, winter and spring, coronaviruses are responsible for 35% of respiratory infections, and during the rest of the year, they are responsible for 15% of respiratory infections (Wright 2010). Coronaviruses are medium sized, single stranded enveloped viruses with a positive sense RNA genome belonging to the Coronaviridae family. Historically, three groups of human and animal coronaviruses have been identified. Group I human coronaviruses (HCoV) include the 229E strain and other related strains. Group II human coronaviruses include the OC43 strain and other related strains. Group III coronaviruses are avian viruses (Greenberg 2011; Wright 2010). A new coronavirus not related to coronaviruses in groups I, II or III was found to be the causative agent of severe acute respiratory syndrome (SARS) (Kahn and McIntosh 2005; Drosten et al. 2003; Kuiken et al. 2003). Coronaviruses received much attention after the SARS epidemic, and in addition to the discovery that the virus causing SARS was from the coronavirus family, two other new coronaviruses were discovered: HCoV-NL63, and HCoV-HKU1 (Rota et al. 2003; Esper et al. 2005; van der Hoek et al. 2004). In 2012 another coronavirus was discovered, Middle East respiratory syndrome coronavirus (MERS-CoV), that causes severe acute respiratory illness and is associated with high mortality rates. While prevalence depends on location, in general coronavirus are thought to be most prevalent during the winter months (Berry et al. 2015). Transmission is via respiratory droplets with an incubation period of 2 to 5 days (La Rosa et al. 2013; Lessler et al. 2009).

Adenovirus

Adenoviruses can cause a variety of clinical syndromes, the most common being respiratory infections, gastroenteritis and conjunctivitis, and rarely cystitis, hepatitis and myocarditis (Ghebremedhin et al. 2014; Lynch et al. 2011). Adenoviruses are double-stranded, non-enveloped DNA viruses that belong to the Adenoviridae family with at least 52 different serotypes, organized into six species A to G. The NxTAG® Respiratory Pathogen Panel uses the hexon protein gene to identify adenovirus. About 1% to 7% of the respiratory infections in adults and 5% to 10% in children are caused by adenoviruses, with serotypes 1 through 7 and 11 being the most common respiratory pathogens in children. Transmission occurs via droplets with infections occurring throughout the year (Lynch et al. 2011). The incubation period for infection ranges from 4 to 8 days (Lessler et al. 2009). Epidemics of adenovirus infection are not common in the general population, but may appear when conditions predispose; for example, when a susceptible population is confined in a high density setting, such as a military base or long-term care facility. Such epidemics tend to occur in winter or early spring (Lynch et al. 2011; Moon 1999).

Human Bocavirus (HBoV)

Human Bocavirus (HBoV) is a recently identified virus in the Parvoviridae family. HBoV is a single-stranded non-enveloped DNA virus (Jartti et al. 2012a) that causes respiratory symptoms including cough, rhinorrhea, fever, and wheezing, and can sometimes also be associated with diarrhea (Mahony 2008; Milder and Arnold 2009; Arnold et al. 2008). Four human bocaviruses, HBoV1 to 4, have been identified but HBoV1 is mainly responsible for the respiratory symptoms (Calvo et al. 2008; Peltola et al. 2013). Bocavirus has a high rate of co-detection with other pathogens (Jartti et al. 2012b). However, HBoV serology studies that also show the presence of HBoV DNA provides evidence that HBoV can cause disease on its own (Karalar et al. 2010; Endo 2007; Soderlund-Venermo et al. 2009). Infections are most common in winter but occur year-round (Jartti et al. 2012b). Little is known on transmission, but with a recent report of hospitalization of a young child with pneumonia with HBoV1 detected in him and his family members, it is likely through respiratory droplets (Jula et al. 2013).

Chlamydophila pneumoniae

Chlamydophila pneumoniae (*C. pneumoniae*) is a member of the *Chlamydiae* family of obligate intracellular bacteria with a biphasic development cycle. *C. pneumoniae* alternates between a highly condensed, non-metabolic extracellular infectious form called the elementary body (EB), and an intracellular, transcriptionally active, non-infectious form called the reticulate body (RB) (Roulis et al. 2013). While the majority of *C. pneumoniae* infections are asymptomatic, approximately 10% of community acquired pneumoniae (CAP) is caused by *C. pneumoniae*. Infection is spread by droplet with an incubation period of 1 to 2 weeks. Symptoms include a slight fever, rhinitis, hoarseness and long-lasting dry cough. Outbreaks are associated with institutions such as schools, long term care homes, and military barracks (Benitez et al. 2012; Choroszy-Krol et al. 2014). *C. pneumoniae* is also found in children with acute lower respiratory tract infection. While infection does occur year round, the majority of infections occur in winter (January to April) (Choroszy-Krol et al. 2014).

Mycoplasma pneumoniae

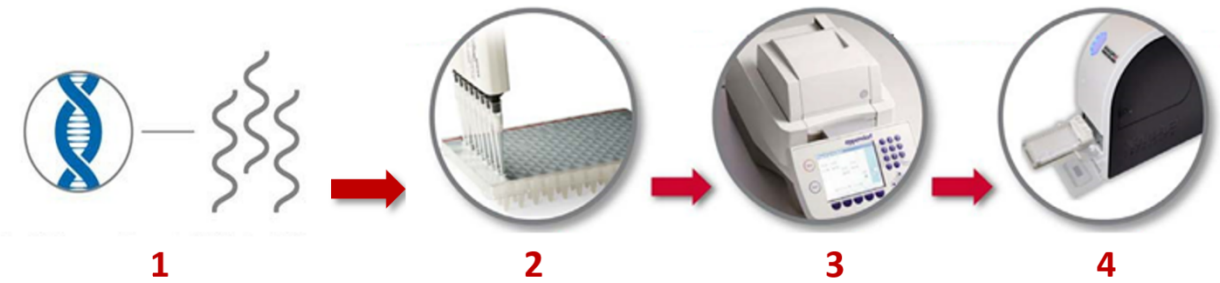
Mycoplasma pneumoniae is a member of the class *Mollicutes*, family *Mycoplasmataceae* and order *Mycoplasmatales*. Bacteria in this class have a small single circular chromosome with a low G+C content, and the permanent lack of a cell wall (Waites and Talkington 2004). *M. pneumoniae*, a common cause of upper and lower respiratory tract infections, is a frequent cause of community acquired pneumonia (CAP) contributing to 40% of infections in children over 5 years of age (Basarab et al. 2014; Atkinson and Waites 2014; Waites and Atkinson 2009). Epidemics occur every 4 to 7 years, thought to be due to introduction of new subtypes, with outbreaks occurring in schools and universities (Atkinson and Waites 2014; Thurman et al. 2009). However, milder presentations of *M. pneumoniae* infection are 20 times more common than CAP with 20% of infections being asymptomatic. The most common type of mild infection is tracheobronchitis (chest cold) which is often associated with upper respiratory tract symptoms. *M. pneumoniae* spreads slowly via respiratory droplets with an average incubation period of 20 to 23 days (Atkinson and Waites 2014; Winchell 2013; Nilsson et al. 2008). *M. pneumoniae* can be shed for long periods (up to 4 months) in respiratory secretions after acute infection (Waites and Talkington 2004; Basarab et al. 2014). Infections can occur during the year but are more common in summer and fall (Winchell 2013).

Principles of the Procedure

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) with the Luminex® proprietary universal tag sorting system on the Luminex platform to detect respiratory pathogen targets. Extracted total nucleic acid is added to pre-plated, Lyophilized Bead Reagents (LBRs), and mixed to resuspend the reaction reagents. The reaction is amplified via RT-PCR and the reaction product undergoes near simultaneous microsphere hybridization within the sealed reaction well. The hybridized, tagged microspheres are then sorted and read on the MAGPIX® instrument. The generated signals are analyzed using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 Assay File for SYNCT™ Software, providing a reliable, qualitative call for each of the 21 targets and internal controls within each reaction well.

Assay Controls

- **Internal Control** - Bacteriophage MS2 is the internal control for the assay. This internal positive control is added to each specimen prior to extraction. This internal control allows the user to ascertain whether the assay is functioning properly. Failure to detect the MS2 control indicates a failure at either the extraction step, the reverse-transcription step, or the PCR step, and may be indicative of the presence of amplification inhibitors, which can lead to false negative results.
- **Positive Controls** - Positive controls are not included in the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay, but are recommended to be included in every run, as a good laboratory practice. External positive controls should be used in accordance with local, state, federal accrediting organizations, as applicable.
- **Negative Amplification Control (No Template Control (NTC))** - The negative amplification control is RNase-free water.
- **Negative Extraction Control (NEC)** – The negative extraction control is the sample collection media that has undergone the entire assay procedure, starting from extraction.



Step 1	Nucleic acid extraction
Step 2	Load extracted nucleic acid to pre-plated test wells
Step 3	Multiplex RT-PCR and hybridization
Step 4	Data acquisition on MAGPIX instrument

Materials Provided

The following table outlines reagents supplied in the kit and their storage conditions. Ensure the kit you are using is for NxTAG® Respiratory Pathogen Panel + SARS-CoV-2.

Table 1. Reagents Supplied with the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Kit

Reagents	Volume for 96 Tests	Storage Conditions
NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Plate	1 - 96-well plate containing 2 Lyophilized Bead Reagents per well	Store at 2°C to 8°C in the re-sealable pouch provided; avoid exposure to light and moisture.
MS2	1.5 mL x 1 vials	Store at -25°C to 8°C.
Foil Seals	8 pieces x 1 case	Store at 2°C to 30°C. Store at 15°C to 30°C after first use.

For a copy of the Safety Data Sheet (SDS), contact Luminex Technical Support.

NOTE: Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Kit lots are identified on the kit carton label.

NOTE: The kit is shipped at 2°C to 30°C. Upon receipt, store the kit at 2°C to 8°C.

NOTE: To avoid exposing the NxTAG RPP + SARS-CoV-2 plate to moisture, do not discard the desiccants included in the resealable pouch.

Software Supplied

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay file for use in the SYNCT™ Software, the MAGPIX® data acquisition protocol, and the package insert will be provided.

Materials Required but not Provided

Recommended Extraction Agents

Choose an extraction system from the list below. The associated reagents and consumables are also required.

- bioMérieux® NucliSENS® easyMAG® System (Product No. 280140) with Generic protocol and associated reagents and consumables
- bioMérieux EMAG® System (Product No. 418591) with Generic protocol and associated reagents and consumables

Equipment

- Computer with:
    - Operating System Microsoft® Windows® 7, 64-bit or Windows 10
    - PC Specifications as stated in the *SYNCT™ Release Notes*
    - SYNCT Software
- Luminex® instrument (MAGPIX®)
    - xPONENT® Software, calibrators, verifiers, controls, and Drive Fluid
- Sonicator bath (Ultrasonic Cleaner, Cole-Parmer®, A-08849-00) or equivalent
- Multichannel pipette or single channel pipette (10 µL to 200 µL)
- PCR cooler rack (Eppendorf® 022510509) or equivalent
- Micronic Pierceable TPE Capmat Black (Cat. No. MP53087) or equivalent for thermal cyclers without adjustable lids
- Thermal Cycler

Consumables

- Optional: EMAG® 1000 µL tips (bioMérieux® Ref. 418922)
- NxTAG® Probe Adjustment Strip (Cat # C000Z0452)
- 96-well Non-Skirted Plate in a clear frame (Cat # C000Z0453) for thermal cyclers that are not compatible with fully-skirted plate
- Skirted Plate (Cat # C000Z0455) (96-well in white frame)

Replacement Materials (if needed)

NOTE: Full foil sheets can be purchased from 4titude, Catalog #: 4ti-0531.

- Foil Seals (Cat # C000Z0454) (8 pieces per case, each piece reseals 3 strips of 8-vessel/strip)

Warnings and Precautions

1. For *in vitro* diagnostic (IVD) use under the FDA Emergency Use Authorization (EUA) only.
2. For prescription (Rx) use only.
3. For professional use only.
4. This test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
5. This test has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2 Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Bocavirus, *Chlamydophila pneumoniae*, and/or *Mycoplasma pneumoniae*, not for any other viruses or pathogens.
6. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
7. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
8. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.
9. Performance characteristics for influenza A were established using specimens obtained during the 2013/2014 and 2014/2015 influenza seasons when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. 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
10. Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases. Use only supplied or specified required consumables to ensure optimal test performance.
11. Care should be taken when handling, storing, and disposing of potentially infectious materials. Suitable barrier protection against potential pathogens is recommended during all stages of use. Gloves and laboratory coats should be worn at all times. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Handle waste disposal in accordance with accepted medical practice and applicable regulations.
12. All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
13. Fresh clean gloves must be worn in each area and must be changed before leaving that area.
14. Do not pipette by mouth.
15. For pre-analytical (sample extraction) steps, use the procedure that is provided with the sample extraction system.
16. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results. Modifications to assay reagents, assay protocol, or instrumentation is not permitted, and are in violation of the product Emergency Use Authorization.

17. Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Lot numbers are identified on the kit label.
18. Handle all samples as if infectious using safe laboratory procedures such as those outlined in CDC/ NIH *Biosafety in Microbiological and Biomedical Laboratories*, and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*.
19. For use with the Luminex® MAGPIX® instrument only.
20. Follow your institution's safety procedures for working with chemicals and handling biological samples.
21. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
22. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at www.luminexcorp.com.

Assay Procedure

Collect Specimen and Extract Nucleic Acid

NOTE: Standard precautions should be taken with regard to sample collection, handling, and storage prior to extraction (refer to the latest edition of the CLSI MM13-A Guideline; and Farkas et al. (1996)).

Extract samples and external controls by either bioMérieux® NucliSENS® easyMAG® System or bioMérieux EMAG® System.

The recommended sample type for the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 is a nasopharyngeal swab in Universal Transport Media (UTM™) or equivalent.

Specimens can be stored between 2°C and 8°C for up to 7 days after collection in Universal Transport Media (UTM™) or equivalent. If the specimen is not going to be tested within 7 days of collection, then it should be stored at ≤-70°C.

Extract Nucleic Acid

1. Briefly vortex to mix the sample.
2. Spike 10 µL of MS2 (internal control) into 200 µL of sample.

   NOTE: The extraction method recommendation for use with this assay is the bioMérieux® NucliSENS® easyMAG® Generic 2.0.1 protocol, and the bioMérieux EMAG® Generic protocol.
3. Use one of the recommended extraction procedures (described below) for the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Assay.

   NOTE: Luminex recommends at least one negative extraction control per extraction batch.
4. Extracted nucleic acid can be refrigerated for 4 hours, if not using within 4 hours store at ≤ -70°C.

Extract Nucleic Acid using the bioMérieux® easyMAG® and EMAG® Systems

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay is validated for use with the bioMérieux easyMAG and EMAG nucleic acid purification systems. Use the parameters provided below.

NOTE: Refer to the manufacturer's instructions for use of bioMérieux easyMAG and EMAG.

To configure the easyMAG, use these parameters:

Table 2. Parameter for the bioMérieux® easyMAG® System with Generic Protocol

Page Name	Parameters	Settings
Define Extraction Request	Sample ID	Enter Sample ID
	Protocol	Generic
	Matrix	Other
	Volume	0.200 mL
	Eluate	110 µL
	Type	Primary
	Priority	Normal or High
Create Run (New Run window)	Run	Enter run name
	Workflow	Select: On-board Lysis Incubation, On-board silica incubation

Table 3. bioMérieux® easyMAG® System Silica Preparation and Addition

Extraction Step	Instructions
Silica Preparation	Dilute easyMAG® silica 1:1 in DNAse/RNAse free water
Silica Addition	Add 100 µL of diluted silica after on-board lysis incubation is complete, pipette mix five times at 1000 µL

To configure the EMAG for use with NxTAG Respiratory Pathogen Panel + SARS-CoV-2, create an NxTAG RPP + SARS-CoV-2 extraction protocol:

Table 4. Parameter for the bioMérieux® EMAG® System with Generic Protocol

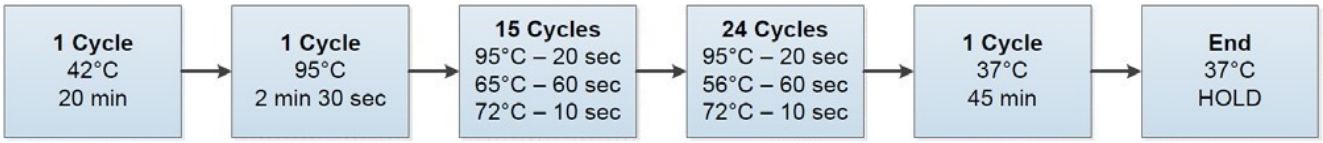
Tab Name	Parameters	Settings		
General	Extraction Method Name	Name the protocol. Example: (NxTAG RPP + SARS-CoV-2)		
	Description	Write a description of the protocol. Example: "Luminex protocol for NxTAG RPP + SARS-CoV-2 sample extraction"		
Input	Off-board Lysis	Off (Do not select)		
	Matrices	Respiratory		
	Valid input volumes	List volume: 210 µL Default volume: 210 µL		
Preparation	Add these items to the **Preparation protocol steps** table in the indicated order	**#**	**Preparation protocol steps**	**Details of selected step**
		1	Samples already prepared:	Do nothing
		2	Distribute reagent bottle to well:	Reagent Bottle: LB (lysis buffer) Volume: 2000 µL
		3	Incubate at room temperature:	Duration: 600 seconds
		4	Transfer silica to well:	Silica Name: Silica Volume: 50 µL
		5	Incubate at room temperature:	Duration 600 seconds
Extraction	Extraction Protocol	Generic		
	Valid Elution Volume	List volume: 110 µL, Default volume: 110 µL		
Eluate Transfer	-	Select: Keep eluates in vessel		
Status	-	Activated		

Program and Preheat Thermal Cycler

NOTE: Perform PCR setup in the pre-PCR area.

Program the following PCR protocol into the thermal cycler with a heated lid (105°C), and pre-heat the thermal cycler to 42°C prior to plate setup:

Figure 1: PCR and Hybridization Conditions



The total thermal cycling run time for NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 should range between 2 hours 15 minutes and 2 hours 45 minutes.

Setup the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Reaction Plate

NOTE: Pre-heat the thermal cycler to 42°C prior to plate setup.

NOTE: Perform PCR setup in the pre-PCR area.

1. If frozen, thaw the extracted nucleic acid samples. Briefly vortex the samples followed by a quick spin to collect the samples to the bottom.
2. Place samples on a chilled PCR cooler block or equivalent.
3. Remove the assay plate from its storage pouch. Place the required number of vessels into the appropriate PCR set-up plate (e.g., skirted plate for Eppendorf® and non-skirted plate for ABI Veriti thermal cycler).

   NOTE: Luminex recommends the first sample be placed in location A1.

   a. Firmly press down on the strips to snap into place, ensuring they are flush with the plate surface.
   b. Return unused vessels to the pouch, seal, and store at recommended storage conditions.

      NOTE: Protect the assay plate from prolonged light exposure.
4. Tap the plate on the benchtop to ensure the Lyophilized Bead Reagents (LBRs) are at the bottom of the vessel.
5. Place the plate on a chilled PCR cooler block or equivalent.
6. Use the end-tabs to peel the clear release liner.

   NOTE: Do not touch the black adhesive.
7. Dispense 35 µL of sample or control to each PCR vessel, by using the pipette tip to pierce the foil at an angle.
   a. Insert the tip a third to halfway down into the vessel.
   b. Dispense the sample into the vessel and wait 1 to 2 seconds while maintaining the pipette tip inside the vessel.
   c. Push the tip all the way to the bottom of the vessel and pipette up and down at least three times to reconstitute the LBRs.
8. Reseal the plate after the sample addition using the precut strips of foil provided. Apply the foil(s) directly on top of the plate and press firmly on and around the wells to ensure a tight seal.

   NOTE: Ensure the foil covers the wells and surrounding black adhesive.

   NOTE: Do not vortex and spin down the plate.

Run Thermal Protocol

Start the Thermal Program

1. Place the foil-sealed plate in the pre-heated thermal cycler and run the protocol.
2. If using a thermal cycler without an adjustable lid, place a micronic pierceable TPE Capmat black or equivalent on top of the sealed plate.

Setup System Software

Import the Data Acquisition Protocol into xPONENT® Software

NOTE: Please refer to the applicable user manual. Ensure the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 data acquisition protocol is saved to a location that is accessible by the xPONENT® Software on the MAGPIX® computer.

If the appropriate protocol is already installed on the computer that controls the Luminex® instrument where the assay is being run, skip the following steps:

1. Log into xPONENT Software.
2. Navigate to the **Protocols** page > **Protocols** tab.
3. Click **Import**.
4. In the **Open** dialog box, browse to the folder where the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 data acquisition protocol is located, and choose the **NxTAG RPP + SARS-CoV-2 T-A[1].lxt2** protocol file. Click **Open**.
5. In the **Imported Protocol File** dialog box, click **OK**. The imported protocol is displayed in the **Installed Protocols** section.

Configure MAGPIX® for Data Acquisition

Prepare the System

NOTE: Please refer to the applicable user manual for software requirements, setup, calibration and verification, and troubleshooting.

NOTE: When setting up xPONENT®, ensure that the Use US regionalization format only option is selected in Admin > CSV Options.

NOTE: Make sure you are using a NxTAG®-enabled MAGPIX® instrument.

1. Log into the xPONENT Software.
2. Perform the **Enhanced Startup Routine** at least once a week along with the required probe sonication.
3. Adjust the sample probe height at least once a week, or as needed.
    a. When adjusting the sample probe height, use the same plate type that will be used when running the NxTAG RPP + SARS-CoV-2 assay plate. Use either the skirted plate or the non-skirted plate (if using an ABI thermal cycler) with the NxTAG Probe Adjustment Strip and one alignment sphere.

        NOTE: Probe height must be re-adjusted if changing between skirted and non-skirted plates.

    b. Save probe height adjustments as **NxTAG Assay Plate**. If prompted to over-write the existing results, click **Yes**.

NOTE: For more information on adjusting the sample probe height, refer to the applicable user manual.

4. Navigate to the **Maintenance** page > **Probe & Heater** tab.
5. Select **ON** under **Plate Heater** and enter **37** in the **Set Temperature** field to heat the MAGPIX® heater plate to 37°C. Click **Apply**.
6. Navigate to the **Maintenance** page > **Cmds & Routines** tab. Click **Eject**. Add the appropriate reagents to the off-plate reagent reservoirs, as specified by the **Post-Batch Routine** indicated in the software. Click **Retract**.

   NOTE: The Post-Batch Routine is included in the assay protocol.

Create Batch in xPONENT® Software

1. Navigate to the **Batches** page > **Batches** tab > click **Create a New Batch from an Existing Protocol**.
2. Choose the **NxTAG RPP + SARS-CoV-2 T-A** protocol in the **Select a Protocol** list.
3. Click **Next**. Select the appropriate wells where the samples will be analyzed and then click **Unknown**. The selected wells are highlighted.
4. Click **Import List** to import a sample list or enter the appropriate Sample ID for each well. Do not change the default **Dilution** settings.

   NOTE: The Sample ID name cannot be duplicated within a Run. Each sample MUST have a unique ID. If you are running replicates or running the same control sample more than once, please make sure you assign a unique Sample ID, for example, by assigning "-1" or "-2" to the end of the proposed Sample ID.
5. Click **Save**. The batch is now saved as a pending batch and ready to run.
6. If running multiple batches on the same plate, create a Multi-Batch.

Create a Multi-Batch in xPONENT® Software

The Multi-batch feature automatically sets the batches side-by-side if space remains on the plate. Ensure that the batches fit on one plate. If space limitations create an overlap, an error message displays. Results for each batch are saved as individual batch files. Batches must be created first, before they can be combined on one plate to create a multi-batch.

NOTE: There is a limit of 96 batches in a multi-batch.

NOTE: You cannot add a batch that forces multiple plates to a multi-batch operation. All batches must use the same plate name.

1. Navigate to the **Batches** page > **Batches** tab **>** click **Create a New Multi-Batch.** The **New Multi-Batch** subtab displays.
   a. If the **Select Pending Batch** dialog box displays, choose the batch you want to add to the new multi-batch list.
   b. Click **OK**.
2. Click **Add** to add a batch. The **Select Pending Batch** dialog box displays.
3. Choose a batch from the available options, including batches newly created.
4. Click **OK**. The selected batch will then display on the plate layout.

   NOTE: After you add each batch, the software automatically adds the next batch to the first well of the next column or row (depending on the plate direction). You can also select a well first, which places the next batch in your chosen location.

   NOTE: If the batches chosen do not fit on the plate, a **Multi-Batch Error** dialog box opens, indicating you must edit one or more of the selected batches.

Acquire Data

Run Batch in xPONENT® Software

1. Navigate to the **Batches** page > **Batches** tab. Choose the pending batch that you want to run.
2. Upon completion of thermal cycling, click **Eject** to place the assay plate on the prepared MAGPIX heater block. Click **Retract** to retract the holder.

   NOTE: Be sure to leave the seal in place.

   NOTE: When placing the plate on the heater block, ensure that the numbers are on the left side and the letters are closest to you.

3. Click **Run** to start acquisition.
4. Verify the information in the warning dialog boxes and click **OK**.

Complete Run in xPONENT® Software

1. When the run is complete, navigate to the **Home** page > **Probe and Heater** tab.
2. Select **OFF** to turn off the heater and click **Eject** to remove the plate from the heater block. Then, click **Retract**.
3. Carefully discard the test vials into a biohazard bag, sealing the bag to avoid aerosolization of the amplicons.
4. If re-using the plate, clean by soaking in a 10% household bleach solution for 15 minutes.
5. Rinse the plate under running tap water to remove the bleach, and air dry on paper towels or wipe with a cloth soaked in 70% alcohol for fast drying, if necessary.

Setup SYNCT™ Software

Install the NxTAG® Module in SYNCT™ Software for the First Time

Ensure that the SYNCT™ Software is on your computer with the NxTAG® module installed. If SYNCT Software is not installed, or the NxTAG module is not installed, then follow the procedures in the *SYNCT Installation Instructions*.

Import the Assay File into SYNCT™ Software

NOTE: Ensure the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay file is saved to a location that is accessible by the SYNCT™ Software.

If you have already imported the correct version of the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 assay file into SYNCT (Assay Code: NRSB, Assay Version A), skip the following steps:

1. Start the SYNCT Software and login with the appropriate SYNCT user.
2. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Assay Management** page.
3. Click **Import Assay** from the Page Action bar at bottom of the page. The **Import File** window displays.

   NOTE: Do NOT double click. SYNCT™ Software requires one click when navigating to the correct file location.

   a. Choose the **Devices** and the **Files**.
   b. Choose the location under **Files** to locate **NxTAG RPP + SARS-CoV-2_NRSB_A.assay** to import, the file name will populate in the **File Name** field.
   c. Click **OK**.

Define Controls and Test Panels

Define a Negative Amplification Control (No Template Control) in SYNCT™ Software

To define a negative amplification control in SYNCT™ Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
   a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
   b. Choose the **NxTAG RPP + SARS-CoV-2** assay in the **Assay** field with the corresponding assay code and version.
   c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
      i. Set the expected result for all tests to **Negative** by selecting the **All Negative** check box.
      ii. Click **Close**.
   d. Click **Save**. The newly defined control displays in the **Controls** window.

Define a Negative Control in SYNCT™ Software

To define a negative control in SYNCT™ Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
   a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
   b. Choose the **NxTAG RPP + SARS-CoV-2** assay in the **Assay** field with the corresponding assay code and version.
   c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
      i. Set the expected result for all tests to **Negative** by selecting the **All Negative** check box.

         NOTE: If the internal control was added to the negative, select Positive as the expected result for the internal control.

      ii. Click **Close**.
   d. Click **Save**. The newly defined control displays in the **Controls** window.

Define an External Positive Control in SYNCT™ Software

NOTE: Name the controls the same name as the controls in xPONENT®, so the control will be automatically defined in the SYNCT™ Software.

To define a external positive control in SYNCT Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
   a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
   b. Choose the **NxTAG RPP + SARS-CoV-2** assay in the **Assay** field with the corresponding assay code and version.
   c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
      i. For tests that are known to be positive in the sample, set the expected result to **Positive**.
      ii. For tests that are known to be negative in the sample, set the expected result to **Negative**.
      iii. If the expected result is unknown for a particular test, select **NA (No Analysis)**.
      iv. Click **Close**.
   d. Click **Save**. The newly defined control displays in the **Controls** window.

Define Test Panels in SYNCT™ Software

For each Order in SYNCT™ Software, you can choose whether a test result is Selected or Masked. Masked test results will not be reported for that sample. If certain subset of tests is ordered regularly, you can pre-define a Test Panel to make the ordering process easier. Then you can select the appropriate Test Panel when editing the Order instead of selecting or masking individual tests.

A default Test Panel that has all the tests selected is provided with the assay.

To define a Test Panel in SYNCT Software within the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Assay Management** page.
2. Choose the **NxTAG RPP + SARS-CoV-2** assay.
3. Click **Assay Options** from the Page Action bar at bottom of the page. The **Assay Options** window displays.
   a. Click the **Test Panels** tab at the top of the window.
   b. Click the **New Panel** button to create a new **Test Panel**. The new **Test Panel** displays within the **Test Panels** section.
   c. By default, all tests are **Selected** for the **Test Panel**. Create a custom **Test Panel** by clicking the **Masked** setting for the appropriate test(s).

      NOTE: Tests with Masked settings chosen will not have test results reported.

   d. Click **Save Changes**.
   e. In the **Messages** dialog box that displays, click **OK**.

Analyze Results in SYNCT™ Software

Create Run from Imported Raw Data in SYNCT™ Software

The Import Raw Data function allows a raw data (CSV) file from xPONENT® Software to be imported.

⚠ Modified output csv data files cannot be used for diagnostics purposes. The integrity of the xPONENT ® CSV file will be checked when the file is imported into SYNCT™. The user will be notified if the file has been modified outside of the system.

To manually import the xPONENT raw data into the SYNCT Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **NxTAG** > **Runs** page.
2. Click **Import Raw Data** from the Page Action bar at bottom of the page. The **Import xPONENT Data** window displays.

   NOTE: Do NOT double click. SYNCT Software requires a single click when navigating to the correct file location.

   a. Choose the **Location** and the **Files**.
   b. Choose the batch file. The **Run Name** field is automatically populated with the Batch name from the xPONENT file.

      NOTE: By default, the Run Name is the same as the batch name imported from the xPONENT file.

   c. Click **OK**. Orders are created for all samples within the imported batch file and can then be edited in SYNCT.

Edit and Review Orders in SYNCT™ Software

After the batch data is imported, an Order is created for each of the samples in the batch file. Review and edit the Orders prior to analyzing the Run.

NOTE: The Sample ID name cannot be duplicated within a Run. Each sample MUST have a unique ID. If you are running replicates or running the same control sample more than once, please make sure you assign a unique Sample ID, for example, by assigning "-1" or "-2" to the end of the proposed Sample ID.

Select multiple orders of the same sample type (sample or control) and edit them at the same time. This is useful when entering kit lot information for all sample orders at the same time, or for applying a Test Panel to multiple orders at the same time. Complete the following in SYNCT™ Software:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **NxTAG** > **Runs** page.
2. Click the "+" sign next to the Run that contains the samples to edit.
3. Select the sample(s) to edit.
4. Click **Edit Orders** from the Page Action bar at bottom of the page.
5. In the window that displays, edit the following information:

   - For **Samples**:

     i. From the **Sample Type** drop-down menu, choose **Sample**.
     ii. If allowed, from the **Test Panels** drop-down menu, choose the appropriate Test Panel OR customize any of the tests listed by clicking **Selected** or **Masked**.
     iii. Update the name of the sample in the **Sample ID** field (Available if a single Order is selected for editing).
     iv. Optionally, include any necessary information in the **Accession ID** and **Requisition Number** fields.

     NOTE: Depending on the SYNCT settings, the Accession ID and Requisition Number may not be visible or you may not have to enter any information within those fields.

     v. Optionally, enter the kit lot number in the **Kit Lot Number** field.

     NOTE: Kit lot numbers are 11 digits separated by a dash. Do not omit the dash when entering the number.

     NOTE: If you enter a Kit Lot Number, you will be required to enter a Lot Expiration date.

     vi. Optionally, click the calendar icon in the **Kit Lot Expiration** field to set the lot expiration date.

     NOTE: Use information provided with your kit for the Kit Lot Number and Kit Lot Expiration.

     vii. Click **OK**.

   - For **Control**:

     i. From the **Sample Type** drop-down menu, choose **Control**.
     ii. Click to choose a pre-defined control to be applied.
     iii. Enter the name of the control in the **Sample ID** field. (Available if a single Order is selected for editing.)
     iv. Optionally, enter the kit lot information in the **Kit Lot Number** field.
     v. Optionally, click the calendar icon in the **Kit Lot Expiration** field to set the lot expiration date.

     NOTE: Use information provided with your kit for the Kit Lot Number and Kit Lot Expiration.

     vi. Click **OK.**

Process Run in SYNCT™ Software

To process the Run in SYNCT™ Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **NxTAG > Runs** page.
2. Select the Sample ID (Run) to process.

3. Click **Process Run** from the Page Action bar at bottom of the page. A dialog box displays, "**Confirm all orders are correct before proceeding. Do you want to continue?**".
4. Click **Yes** to proceed with processing the Run.
5. Once the Run has completed processing, the Run is removed from the **NxTAG Run** view. The results of the Run can be found by clicking the **Results** icon from the **System Navigation Menu** and locating the processed Run from the list.

Result Call Definitions

For a general description of Results page functionality, please refer to the *SYNCT™ Software User Manual*.

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Results** > **Results** page.
2. Click the "+" sign next to the Run Results you want to see a Status for.

   - The **Status** column indicates whether there are Errors, Warnings, Info messages, or user comments for a sample. Click the ▷ in the **Status** column to display the messages in the sample row. The **Status** column will display a ⊗ if a sample has an error. If there are no messages for the sample, the ▷ will not appear.
   - The **Alert** column indicates if any test has a positive result. If the result is positive the **Alert** column will display a ✚ for that sample.
   - The **Alert** column indicates if a control has failed. If the control failed, the **Alert** column will display a red exclamation mark for that control.
   - The **Result** column displays the summary result for the sample. To see individual results for each test, click ▷ next to the summary results in the **Result** column. The results are shown grouped by result type in the sample row.

   The following results can appear for samples:

Result Column	Meaning
Invalid	Any target that has an invalid result. Some targets may have valid positive or negative results. Expand the Result column to see the results for the individual targets.
Target1 Positive, Target2 Positive	The specified target has a positive result. A maximum of two positive targets will be listed.
Positive Detected	More than two targets have a positive result.
Negative	All targets are negative.

The following results can appear for controls:

Result Column	Meaning
Pass	All target results match the expected results.
Fail	Any target result does not match the expected result.
Invalid	If all negative controls failed due to instrument error or well not read, then the positive control will be Invalid.

Report Type Definitions

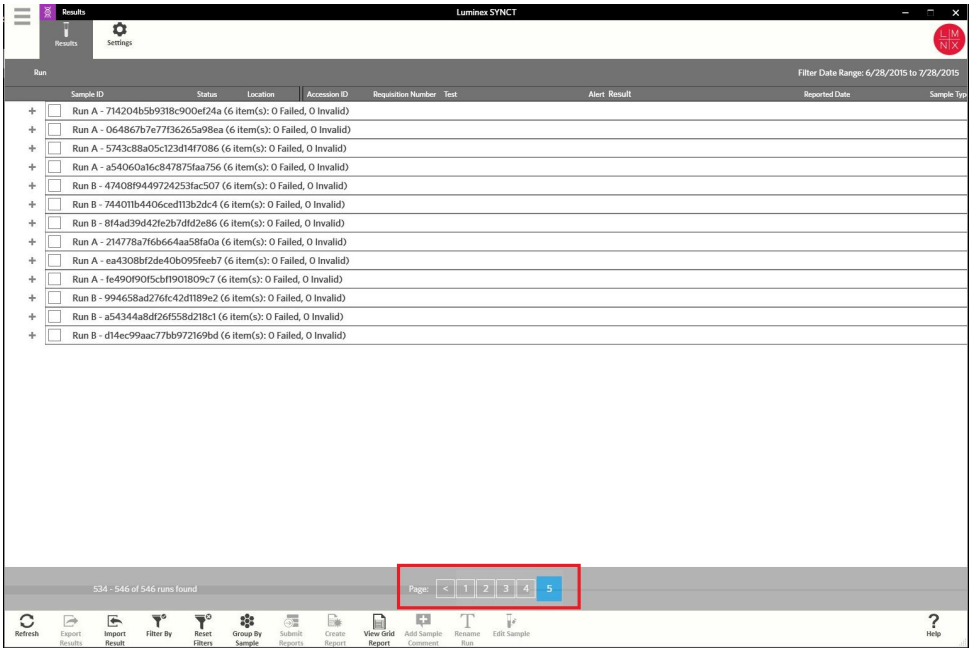
The following reports are available for the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay:

Report Title	Summary of Contents
Clinical Summary	Shows the result for each target for a sample.
Sample Details	Shows the result, calculated signal value, and threshold used to determine the result for each target for a sample.
Control Summary	Shows the expected result, and pass or fail result, for each target for a control.
Control Details	Shows the expected result, pass or fail result, and calculated signal for each target for a control.
Run Report	Shows a summary result for each sample that includes all positive tests.
Run Details	Contains a Run summary, Sample Details for each sample (with optional graph), and details for each selected target (with optional graph).

View Results in SYNCT™ Software

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Results** > **Results** page.

2. When there are multiple pages of results in SYNCT™ Software, page arrows and numbers will display at the bottom of the screen. Click the left and right arrows to scroll through the pages of results or, if you know what page the results are on, click the page number.



Create and Print a Report in SYNCT™ Software

To create a report, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Results** > **Results** page.
2. Select the Run or samples that the report is to be generated for.
3. Click **Create Report** from the Page Action bar at bottom of the page. The **Generate Reports** window displays.

   NOTE: You can select one sample in order to view the report, however the report may have results from other samples in it. You can also export to a chosen location and print the report.

4. Choose the type of report to be created from the options provided. The report displays in a separate window.

   NOTE: Reports generated can have a customized header.

5. In the Report window, click **Print Report** to print the report. The **Print** dialog box will display.
   a. Choose the printer and print settings, then click **Print**.

Interpretation of Results

Run controls will be reported as Pass, Fail or Invalid as detailed in the Result Call Definitions section. The recommended action for Invalid or Failed controls can be found in the Troubleshoot section below.

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay detects two genes in SARS-CoV-2, the ORF1ab gene and the M gene. Detection of either gene is sufficient to call a SARS-CoV-2 Positive.

Table 5. Interpretation of SARS-CoV-2 Results

SARS-CoV-2 Analytes	Other Analytes Detected	Internal Control Detected	SARS-CoV-2 Call	Internal Control Call
ORF1ab or M detected	Yes or No	Yes	Positive	Pass
	Yes or No	No	Positive	NA
Both ORF1ab and M detected	Yes or No	Yes	Positive	Pass
	Yes or No	No	Positive	NA
ORF1ab or M invalid and the other is detected	Yes or No	Yes	Positive	Pass
	Yes or No	No	Positive	NA
ORF1ab or M invalid and the other is not detected	Yes or No	Yes	Invalid	Pass
	Yes	No	Invalid	NA
	No	No	Invalid	Fail
ORF1ab and M not detected	Yes or No	Yes	Negative	Pass
	Yes	No	Negative	NA
	No	No	Invalid	Fail

NA: The Internal Control call is NA if the Internal Control is not detected and one or more target is detected.

Table 6. Interpretation of Influenza A Results

Final Result	Influenza A	H1-A (H1)	H1-B (2009H1N1)	H3	Required Follow-Up
Influenza A Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	Positive	Positive	Negative	Negative	None
	Positive	Positive	Positive	Negative	
	Positive	Negative	Positive	Negative	
	Negative*	Positive	Negative	Negative	
	Negative*	Positive	Positive	Negative	
	Negative*	Negative	Positive	Negative	

Final Result	Influenza A	H1-A (H1)	H1-B (2009H1N1)	H3	Required Follow-Up
Influenza A H3	Positive	Negative	Negative	Positive	None
	Negative*	Negative	Negative	Positive	
Influenza A H1 and Influenza A H3	Positive	Positive	Negative	Positive	None
	Positive	Negative	Positive	Positive	
	Negative*	Positive	Negative	Positive	
	Negative*	Negative	Positive	Positive	
Influenza A (no subtype detected)	Positive	Negative	Negative	Negative	See below

* Detection of Influenza A H1 Influenza A 2009 H1N1, or Influenza A H3 subtypes without an Influenza A "Positive" result may occur at low titer of the virus in the specimen or may indicate a false positive due to contamination. The result could also indicate potential genetic mutations in the Matrix protein gene among circulating seasonal Influenza A viruses.

NOTE: The Influenza A H1 and Influenza A 2009 H1N1 have been combined into a single call because the Influenza A 2009 H1N1 strain is now considered to be the seasonal strain.

Influenza A (no subtype detected)

If the Influenza A analyte is positive, but none of the H1 or 2009 H1N1 and H1-A (probing for H1), H1-B (probing for 2009 H1N1) and H3 subtyping analytes are positive, the interpretation is Influenza A positive, no subtype detected. This result may occur if the titer of the virus in the specimen is low or in the presence of a novel Influenza A strain. In either case, the sample in question should be re-extracted and retested by the device. If the retest provides the same result for influenza A (no subtype detected), contact local or state public health authorities for confirmatory testing.

Internal Control (not detected)

If the internal control is reported "NA" on the SYNCT-processed results, any targets detected will be reported as positive. No action is required from the user.

Human Bocavirus

Currently available evidence suggests that Human Bocavirus causes respiratory illness primarily in young children and possibly the immunosuppressed. The positive predictive value of Bocavirus as a cause of respiratory illness after detection in adults and older children has not been established.

Troubleshoot

Re-Test Recommendations Prior to Data Acquisition

Thermal Cycler Error: If an error in the thermal cycler program is noticed after a particular step is initiated, re-test the samples.

Re-Test Recommendations After Data Acquisition

Under certain circumstances, data analysis software will generate a target call of "Invalid" with associated error message(s) for one or more samples in a plate. These scenarios are summarized (with re-test recommendations) below.

Re-test Recommendations for Invalid Results

Scenarios are summarized (with re-test recommendations) in the table below.

Table 7. Invalid Results

Software Result and Messages	Problem	Possible Cause(s)	Recommendation(s)
Result: Invalid *Message*: "<Target Name>: non- specific signal detected in control sample"	An unexpected target was detected in a control sample.	Contamination may have occurred during extraction, with extraction reagents, during sample addition, or the internal control was added to the negative extraction control.	Re-extract the samples, including the negative extraction control with new (un-used) reagents.
Result: Invalid *Message:* "Run failed. All negative control samples have failed"	An instrument error occurred and all samples identified as negative controls are invalid.	Refer to the applicable user manual for possible causes.	Re-run the samples.
Result: Invalid *Message:* "<Target Name>: invalid value encountered" OR "<Target Name>: low bead count"	The sample probe failed to acquire enough of the sample.	Low sample volume; sample probe height adjustment was not completed successfully. Failed to fully re-suspend Lyophilized Bead Reagents.	Repeat sample probe height adjustment procedure. Re-run the sample. Ensure the Lyophilized Bead Reagents were fully re-suspended.

Software Result and Messages	Problem	Possible Cause(s)	Recommendation(s)
Result: Invalid *Message*: "<Target Name>: invalid negative control value"	Failed to acquire enough of target signal within all negative control samples.	Sample probe height was not completed successfully. Failed to fully re-suspend Lyophilized Bead Reagents.	Re-extract and re-run samples since you cannot rule out contamination for this target.
Result: Invalid *Message*: "Inconclusive results based on abnormal signals"	Background cannot be calculated as multiple targets have abnormal signals.	Contamination may have occurred during extraction, during sample addition, or instrument failure.	Re-extract and re-run the sample.
Result: Invalid *Message*: "Inconclusive results based on abnormal number of positive signals"	More than 7 positive signals were detected in a sample.	Contamination may have occurred during extraction, with extraction reagents, or during sample addition.	Re-extract the samples, including negative extraction control with new (un-used) reagents.
Result: Invalid *Message*: "This well was not read by the Luminex instrument."	No signal is detected.	Instrument failed or user terminated during data acquisition or extraction failure.	Re-extract and re-run the sample.
Result: Fail *Message*: "Control failed: <Target name> result did not match expected result" OR "<Target Name>: non-specific signal detected"	Unexpected target call in the control.	Wrong control samples were used or Extraction failure or error occurred during extraction or sample addition.	Re-extract and re-run the sample.

Resolve Low Bead Count

Table 8. Low Bead Count

Software Result and Messages	Problem	Possible Cause(s)	Recommendations
Result: Invalid *Message*: "Internal Control failed."	Low Bead Count	An insufficient number of beads were aspirated by the MAGPIX® instrument or the beads aggregated in the instrument, preventing an accurate count.	Sonicate and clean the MAGPIX sample probe. Ensure the enhanced startup routine and post-batch cleaning routines are being performed.

Limitations

1. Performance of this test has only been established in nasopharyngeal swab specimens.
2. This device may not be able to differentiate newly emerging Influenza A subtypes.
3. Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
4. All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
5. The detection of pathogen nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
6. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
7. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
8. There is a risk of false negative values due to the presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
9. A specimen yielding a negative result may contain respiratory pathogens not probed by the assay.
10. Positive influenza results obtained in a patient who received FluMist® prior to sample collection may be due to detection of influenza viruses in the vaccine and may mask a true positive result due to infection by one or more of these viruses.
11. The performance of the assay has not been established in individuals who received nasally administered Influenza A vaccine.
12. The performance of this device has not been assessed in a population vaccinated against COVID-19.
13. The clinical performance for SARS-CoV-2 has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

14. The performance of this assay was not established in immunocompromised patients.
15. The performance of the NxTAG® Respiratory Pathogen Panel was established during the 2013/2014 and the 2014/2015 season. The performance for some viruses and subtypes may vary depending on the prevalence and population tested.
16. The performance of the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 was confirmed during the 2019/2020 season. The performance for some viruses and subtypes may vary depending on the prevalence and population tested.
17. Due to the genetic similarity between human Rhinovirus and Enterovirus, the assay cannot reliably differentiate them. A positive NxTAG Respiratory Pathogen Panel + SARS-CoV-2 Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g., cell culture or sequence analysis).
18. Performance characteristics for *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* were established primarily using contrived specimens.
19. This test cannot rule out infections caused by other viral or bacterial pathogens not present on this panel.
20. Coronavirus 229E may produce false positive Influenza H1 results.
21. Parainfluenza virus Type 2 may produce false positive Influenza H3 results.
22. *In silico* analysis revealed some SARS-coronavirus Urbani strains and the isolates Sin_WNV and CUHK-L2 may produce false positive SARS-CoV-2 results.
23. Other non-2009 H1 Influenza viruses have the potential to give a false positive call for Coronavirus 229E.
24. Some strains of Enterovirus D68 may produce false positive results for Influenza A H3 above 1.00E+03 $TCID_{50}$/mL.
25. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
26. This device has been evaluated for use with human specimen material only.
27. The performance of this device has not been evaluated for patients without signs and symptoms of infection.
28. The performance of this device has not been evaluated for monitoring treatment of infection.

Conditions of Authorization for Laboratory

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: *https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas*.

However, to assist clinical laboratories using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2, the relevant Conditions of Authorization are listed below and are required to be met by laboratories performing the EUA test:

1. Authorized laboratories* using NxTAG Respiratory Pathogen Panel + SARS-CoV-2 must include test result reports and all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 must use the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your NxTAG Respiratory Pathogen Panel + SARS-CoV-2 product are not permitted.
3. Authorized laboratories using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

4. Authorized laboratories using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories must collect information on the performance of NxTAG Respiratory Pathogen Panel + SARS-CoV-2 and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: *CDRH-EUA-Reporting@fda.hhs.gov*) and Luminex Corporation, Inc. (via email: *support@luminexcorp.com*) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 must be appropriately trained in RT- PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your productNxTAG Respiratory Pathogen Panel + SARS-CoV-2 in accordance with the authorized labeling.
7. Luminex, authorized distributors, and authorized laboratories using the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

* The letter of authorization refers to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests" as "authorized laboratories."

Performance Characteristics

NxTAG® Respiratory Pathogen Panel and NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

The formulation NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 is identical to the NxTAG Respiratory Pathogen Panel (Catalogue# I051C0447) with the exception of the additional primers required for the detection of SARS-CoV-2. No changes have been made to the existing NxTAG Respiratory Pathogen Panel primers, reaction conditions, workflow or software thresholds. Analytical and clinical performance studies were conducted to establish the performance of the SARS-CoV-2 target and confirm the NxTAG Respiratory Pathogen Panel performance characteristics are still applicable to the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 assay.

Clinical Performance

Clinical Performance of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

The clinical performance of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Assay was evaluated using leftover, de-identified, and blinded nasopharyngeal swabs (NPS) pre-selected clinical specimens collected in Universal Transport Media (UTM™) or equivalent. The pre-selected positive specimens were previously characterized by the standard of care method at the collection site (various molecular assays) and then confirmed by a molecular method prior to testing on NxTAG Respiratory Pathogen Panel + SARS-CoV-2 Assay. Discordant specimens, where the NxTAG Respiratory Pathogen Panel + SARS-CoV-2 Assay result differed from the comparator result, were further assessed by PCR followed by bi-directional sequencing.

The SARS-CoV-2 positive NPS specimens were obtained from three (3) geographically distinct laboratories in the United States (*Table 9*).

Table 9. SARS-CoV-2 Specimen Sources

Site	Location	Positive Specimens Tested
Site 05	Liverpool, NY	10
Site 06	Milwaukee, WI	10
Site 07	Galveston, TX	10

Thirty (30) SARS-CoV-2 positive clinical specimens collected between May 2020 and October 2020 were confirmed as positive by a FDA EUA authorized SARS-CoV-2 molecular test and then tested on the NxTAG RPP + SARS-CoV-2 Assay. In addition, negative specimens, collected prior to the SARS-CoV-2 pandemic, from November 2016 to March 2019, expected to be negative for SARS-CoV-2, were tested by NxTAG RPP + SARS-CoV-2 and NxTAG RPP assays for the qualitative detection of microorganisms associated with common upper respiratory tract infection. Results of the clinical specimens testing for the SARS-CoV-2 target shown in *Table 10* demonstrate a Positive Percent Agreement (PPA) of 100% and Negative Percent Agreement (NPA) of 100%.

Table 10. Clinical Performance for SARS-CoV-2 Specimens

Sample Type/Collection Method	# of Samples Tested	NxTAG RPP + SARS-CoV-2			SARS-CoV-2 Result	% Agreement with Reference Method (PPA/NPA)
		SARS-CoV-2 ORF1ab	SARS-CoV-2 M gene	SARS-CoV-2 Final Result		
SARS-CoV-2 Positive	30	27/30	29/30	30/30	Positive	100%
SARS-CoV-2 Negative*	227	227/227	227/227	227/227	Negative	100%

* Negative samples for SARS-CoV-2 were assumed negative because they were collected prior to the pandemic and were not tested on the comparator method.

For non-SARS-CoV-2 targets, a total of 227 archived clinical specimens were confirmed positive by the NxTAG RPP panel and tested on the NxTAG RPP + SARS-CoV-2Assay. Clinical specimens' comparative analysis results are summarized in *Table 11*.

Table 11. Clinical Performance of NxTAG® RPP + SARS-CoV-2 Assay

Organism	TP / (TP+FN)	PPA%	95% CI	TN / (TN+FP)	NPA%	95% CI	Total Count
Adenovirus	9/9	100.0%	70% - 100%	218/218	100.0%	98%-100%	227
Human Coronavirus 229E	9/9	100.0%	70%-100%	218/218	100.0%	98%-100%	227

Organism	TP / (TP+FN)	PPA%	95% CI	TN / (TN+FP)	NPA%	95% CI	Total Count
Human Coronavirus HKU1	9/9	100.0%	70%-100%	218/218	100.0%	98%-100%	227
Human Coronavirus NL63	14/14	100.0%	78%-100%	213/213507/507	100.0%	98%-100%	227
Human Coronavirus OC43	12/12	100.0%	76%-100%	215/215	100.0%	98%-100%	227
Human Metapnumovirus	10/10	100.0%	72%-100%	217/217	100.0%	98%-100%	227
Human Bocavirus	10/10	100.0%	72%-100%	217/217	100.0%	98%-100%	227
Influenza A	23/23	100.0%	86%-100%	204/204	100.0%	98%-100%	227
Influenza A H1	11/11	100.0%	74%-100%	216/216	100.0%	98%-100%	227
Influenza A H3	12/12	100.0%	76%-100%	215/215	100.0%	98%-100%	227
Influenza B	11/11	100.0%	74%-100%	216/216	100.0%	98%-100%	227
Parainfluenza 1	10/10	100.0%	72%-100%	217/217	100.0%	98%-100%	227
Parainfluenza 2	10/10	100.0%	72%-100%	217/217	100.0%	98%-100%	227
Parainfluenza 3	11/11	100.0%	74%-100%	216/216	100.0%	98%-100%	227
Parainfluenza 4	15/15	100.0%	80%-100%	212/212	100.0%	98%-100%	227
Respiratory Syncytial Virus A	10/10	100.0%	72%-100%	217/217	100.0%	98%-100%	227
Respiratory Syncytial Virus B	21/21	100.0%	85%-100%	206/206	100.0%	98%-100%	227
Rhinovirus/Enterovirus	17/17	100.0%	82%-100%	209/210*	99.5%	98%-100%	227
Chlamydophila pneumoniae	8/9†	88.9%	57%-98%	218/218	100.0%	98%-100%	227
Mycoplasma pneumoniae	12/12	100.0%	76%-100%	215/215	100.0%	98%-100%	227

* The single FP Rhinovirus/Enterovirus was negative by PCR followed by bi-directional sequencing.

† The single FN *C. pneumoniae* was detected by NxTAG RPP + SARS-CoV-2 upon re-testing, for 100% agreement.

Analytical Performance

Limit of Detection (LoD) of SARS-CoV-2 Tested with NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

The Limit of Detection for SARS-CoV-2 in the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Assay was assessed by testing a serial dilution of heat inactivated SARS-CoV-2 culture fluid (ATCC VR-1986HK, Heat-Inactivated Virus) in pooled negative nasopharyngeal specimens (Negative Clinical Matrix, NCM). The LoD titer for SARS-CoV-2 was defined as the lowest concentration at which ≥95% (≥19/20) of samples tested generated positive calls. The LoD of the SARS-CoV-2 target in the NxTAG RPP + SARS-CoV-2 Assay is 500 copies/mL.

The LoD for each of the NxTAG RPP + SARS-CoV-2 targets that are also targets of NxTAG Respiratory Pathogen Panel was confirmed in contrived samples prepared in NCM. The LoD concentration established for NxTAG RPP was tested in 20 replicates with NxTAG RPP + SARS-CoV-2 and the LoD was confirmed if ≥95% (≥19/20) of the replicates generated positive calls. When <95% of the replicates generated positive calls, the analyte was tested at 2X the established LoD in 20 replicates. Twenty-two (22) strains were confirmed at 1x LoD in multi-analyte samples and 6 strains (identified by a '*' in *Table 12*) were confirmed at 2x LoD in a single analyte samples.

The confirmed LoD of all analytes in the NxTAG RPP + SARS-CoV-2 is within 2x to that of the NxTAG Respiratory Pathogen Panel. The LoD of the NxTAG RPP + SARS-CoV-2 are listed in *Table 12*.

Table 12. Summary of Confirmed LoD for Targets Detected by the NxTAG® RPP + SARS-CoV-2

Analyte	Strain	LoD Concentration	Detection with NxTAG RPP + SARS-CoV-2
Influenza A Matrix	A/Brisbane/59/07	3.08E+00 $TCID_{50}$/mL	20/20
	A/SwineNY/03/2009	5.53E-01 $TCID_{50}$/mL	20/20
	A/Wisconsin/67/05	5.00E-01 $TCID_{50}$/mL*	20/20
Influenza A H1 Subtype	A/Brisbane/59/07	3.08E+00 $TCID_{50}$/mL	20/20
	A/SwineNY/03/2009	5.53E-01 $TCID_{50}$/mL	20/20
Influenza A H3 Subtype	A/Wisconsin/67/05	9.36E-02 $TCID_{50}$/mL	20/20
Influenza B	B/Florida/04/2006	5.81E-01 $TCID_{50}$/mL	19/20
Respiratory Syncytial Virus A	A2	2.15E+00 $TCID_{50}$/mL	19/20
Respiratory Syncytial Virus B	18537	1.36E+00 $TCID_{50}$/mL	20/20
SARS-CoV-2	USA-WA1/2020	5.00E+02 copies/mL†	20/20
Coronavirus 229E	VR-740 (ATCC)	1.07E-02 $TCID_{50}$/mL	20/20

Analyte	Strain	LoD Concentration	Detection with NxTAG RPP + SARS-CoV-2
Coronavirus OC43	Betacoronavirus 1 - VR-1558 (ATCC)	7.15E-02 $TCID_{50}$/mL	19/20
Coronavirus NL63	0810228CF (Zeptometrix)	6.74E-03 $TCID_{50}$/mL*	20/20
Coronavirus HKU1	Clinical Specimen	1.57E+04 Copies/mL	19/20
Human Metapneumovirus	Human Metapneumovirus	1.38E-01 $TCID_{50}$/mL	19/20
Rhinovirus/Enterovirus	Rhinovirus type 1A	5.18E-01 $TCID_{50}$/mL	20/20
	Enterovirus D68	3.34E+00 $TCID_{50}$/mL	20/20
Adenovirus	C, type 1	3.25E+00 $TCID_{50}$/mL	20/20
	B, type 14	1.52E-01 $TCID_{50}$/mL	20/20
	E, type 4	1.38E-01 $TCID_{50}$/mL*	20/20
Parainfluenza 1	C35	2.82E+01 $TCID_{50}$/mL	20/20
Parainfluenza 2	Greer	5.35E-01 $TCID_{50}$/mL	19/20
Parainfluenza 3	C 243	3.22E+01 $TCID_{50}$/mL*	20/20
Parainfluenza 4A	Type 4A	5.09E+00 $TCID_{50}$/mL*	20/20
Parainfluenza 4B	CH 19503	6.09E-01 $TCID_{50}$/mL	20/20
Human Bocavirus	Type 1	3.91E+02 Copies/mL	19/20
Chlamydophila pneumoniae	*Chlamydophila pneumoniae*	1.29E-01 $TCID_{50}$/mL*	20/20
Mycoplasma pneumoniae	*Mycoplasma pneumoniae*	1.42E+02 CCU/mL	20/20

* The confirmed LoD concentration is 2X the claimed LoD for NxTAG RPP.

† Concentration of viral RNA determined by Droplet Digital™ PCR (ddPCR™) Technology at supplier.

The composition of multi-analyte samples used for LoD confirmation is listed in *Table 13*

Table 13. Multi-Analyte Samples Tested for LoD Confirmation

MA#	Analyte 1	Analyte 2	Analyte 3
MA1	Influenza A H1	Rhinovirus	Respiratory Syncytial Virus A
MA2	Influenza A H3 (matrix)	Adenovirus C	N/A
MA3	Influenza A 2009 H1N1	Parainfluenza 1	*Chlamydophila pneumoniae*
MA4	Influenza A H3 (Subtype)	Respiratory Syncytial Virus B	Human Bocavirus
MA5	Parainfluenza 3	Coronavirus OC43	N/A
MA6	Influenza B	Parainfluenza 4A	*Mycoplasma pneumoniae*
MA7	Coronavirus NL63	Human Metapneumovirus	Coronavirus HKU1
MA8	Parainfluenza 4B	Parainfluenza 2	Coronavirus 229E
MA9	Adenovirus B	Enterovirus D68	N/A
MA10	Adenovirus E	N/A	N/A

In Silico Analysis (Reactivity and Cross-Reactivity)

SARS-CoV-2 *In Silico* Inclusivity (Reactivity) in NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

In silico inclusivity analyses of the oligonucleotide (oligo) sequences for the SARS-CoV-2 ORF1ab and M gene sets were performed against all SARS-CoV-2 sequences available in the GISAID database as of January 3, 2021. The analysis included 230,623 sequences in the amplicon regions of the ORF1ab and M gene oligo sets, including sequences of the United Kingdom strain (B.1.1.7 lineage) and the South Africa strain (501Y.V2 variant). Based on *in silico* analysis of the percent homology of each oligo sequence to its binding region on each SARS-CoV-2 sequence, it is predicted that NxTAG RPP + SARS-CoV-2 will detect all analyzed SARS-CoV-2 sequences from the GISAID database as of January 3, 2021.

SARS-CoV-2 *In Silico* Cross-Reactivity in NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

An *in silico* cross-reactivity analysis was performed with all primer and probe sequences in NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 against sequences from GenBank nt database available as of May 13, 2020 for organisms listed in *Table 14*. Based on *in silico* analysis, it is predicted that the assay may cross-react with some strains of SARS-coronavirus but not cross-react with any other organisms listed in *Table 14*. The risk of false positives relating to SARS-coronavirus cross-reactivity is minimal due to the very low presence of SARS-coronavirus.

Results from the *in silico* cross-reactivity analysis showed the organisms in *Table 14* with oligo-hit sequence homology ≥80% are *Candida albicans*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, SARS-coronavirus, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. With the exception of SARS-coronavirus, the other seven non-target organisms have only one or two oligo components (forward primer, reverse primer, or probe) with ≥80% homology; as such, there is no anticipated non-specific detection of these non-target organisms because hybridization and extension of all three oligo components are required to generate a detection signal. For SARS-coronavirus, there are some strains where the SARS-CoV-2 M gene oligos have ≥80% homology for all three oligo components. It is predicted that the assay may cross-react with some strains of SARS-coronavirus.

Table 14. Organisms Assessed in *In Silico* Cross-Reactivity Analysis

Human coronavirus 229E	Respiratory syncytial virus A
Human coronavirus OC43	Respiratory syncytial virus B
Human coronavirus HKU1	Rhinovirus
Human coronavirus NL63	*Chlamydia pneumoniae*
SARS-coronavirus	*Haemophilus influenzae*
MERS-coronavirus	*Legionella pneumophila*
Adenovirus	*Mycobacterium tuberculosis*
Human Metapneumovirus (hMPV)	*Streptococcus pneumoniae*
Parainfluenza virus 1	*Streptococcus pyogenes*
Parainfluenza virus 2	*Bordetella pertussis*
Parainfluenza virus 3	*Mycoplasma pneumoniae*
Parainfluenza virus 4	*Pneumocystis jirovecii (PJP)*
Influenza A	*Candida albicans*
Influenza B	*Pseudomonas aeruginosa*
Enterovirus	*Staphylococcus epidermis*
Human bocavirus	*Streptococcus salivarius*
SARS-CoV-2	

Reactivity

SARS-CoV-2 Reactivity Assessed with NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

The analytical reactivity for SARS-CoV-2 in the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay was assessed by both laboratory wet testing and *in silico* analysis. Laboratory testing consisted of four strains of SARS-CoV-2 tested at ≤ 3x LoD in pooled negative nasopharyngeal swabs clinical specimens in Universal Transport Media (UTM™). Heat inactivated strains were processed as per protocol while purified RNA strains were diluted to their target concentration with eluate from a pooled negative clinical matrix extraction and added directly to the assay. All tested strains of SARS-CoV-2 were detected at ≤ 3x LoD.

Table 15. SARS-CoV-2 Analytical Reactivity in the NxTAG® RPP + SARS-CoV-2 Assay

Organism	Strain	Source	Concentration	Detection
SARS-CoV-2	USA-WA1/2020	ATCC, VR-1986HK, Heat-Inactivated Virus	4.77E+02 copies/mL	3/3 100%
SARS-CoV-2	USA-WA1/2020	ZeptoMetrix, 0810587CFHI, Heat-Inactivated Virus	1.50E+03 copies/mL	3/3 100%
SARS-CoV-2	2019-nCoV/Italy-INMI1	European Virus Archive, 008N-03894, purified RNA	1.50E+03 copies/mL*	3/3 100%
SARS-CoV-2	BavPat1/2020	European Virus Archive, 026N-03889, purified RNA	1.50E+03 copies/mL*	3/3 100%
SARS-CoV-2	Hong Kong/ VM200001061/2020	ZeptoMetrix, 0810590CFHI, Heat Inactivated	1.50+03 copies/mL	3/3 100%

* Calculated concentration of RNA in the sample pre-extraction.

Cross-Reactivity

SARS-CoV-2 Cross-Reactivity Tested with NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

Potential cross-reactivity was assessed with pathogens that cause respiratory infections or may be found in respiratory specimens. Cross-reactivity was evaluated by spiking cultured organisms into pooled negative nasopharyngeal swabs clinical specimens in Universal Transport Media (UTM™) to reach specified final concentrations. Viral targets were prepared to reach a concentration at 1.00E+05 $TCID_{50}$/mL or 1.00E+06 copies/mL while bacterial targets were prepared to reach a concentration at 1.00E+06 CFU/mL or CCU/mL or at the highest concentration possible based on the organism stock concentration. Three replicates of each pathogen were tested with the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay. None of the pathogens included in this study cross-react with the targets probed by NxTAG RPP + SARS-CoV-2 assay, with the exception of one strain of Enterovirus D68 (US/IL/14-18952, ATCC VR-1824) which generated Influenza A H3 false positive calls when tested at 1.00E+05 $TCID_{50}$/mL. No false positive calls occurred when tested at 1.00E+03 $TCID_{50}$/mL. Other Enterovirus strains do not produce this cross-reaction. Additionally, *in silico* analysis does not predict a cross-reaction to occur between Enterovirus and Influenza A H3.

Table 16. SARS-CoV-2 Cross-Reactivity in the NxTAG® RPP + SARS-CoV-2 Assay

Organism	Strain	Source	Concentration	SARS-CoV-2 Negativity (#negative/ #tested)
Bordetella pertussis	A639	ZeptoMetrix, 0801459	1.00E+06 CFU/mL	3/3
Candida Albicans	Z006	ZeptoMetrix, 0801504	1.00E+06 CFU/mL	3/3
Haemophilus influenzae	type b; Minn A	ZeptoMetrix, 0801680	1.00E+06 CFU/mL	3/3
Mycobacterium tuberculosis	H37Ra	ZeptoMetrix, 0801660	1.00E+06 CFU/mL	3/3
Pneumocystis jirovecii (PJP)	Pneumocystis jiroveci-S. cerevisiae Recombinant	ZeptoMetrix, 0801698	1.00E+06 CFU/mL	3/3
Pseudomonas aeruginosa	Clinical isolate	ZeptoMetrix, 0801519	1.00E+06 CFU/mL	3/3
Staphylococcus epidermidis (MRSE)	MRSE, RP62A	ZeptoMetrix, 0801651	1.00E+06 CFU/mL	3/3
Streptococcus pneumoniae	Zo22, 19F	ZeptoMetrix, 0801439	1.00E+06 CFU/mL	3/3
Streptococcus pyogenes	Z018F	ZeptoMetrix, 0801512	1.00E+06 CFU/mL	3/3
Streptococcus salivarius	Z127	ZeptoMetrix, 0801896	1.00E+06 CFU/mL	3/3

Organism	Strain	Source	Concentration	SARS-CoV-2 Negativity (#negative/ #tested)
Human Coronavirus OC43	OC43 (Betacoronavirus 1)	ATCC, VR-1558	1.00E+05 $TCID_{50}$/mL	3/3
Human Coronavirus NL63	NL63	ZeptoMetrix, 0810228CF	1.00E+05 $TCID_{50}$/mL	3/3
Human Coronavirus HKU1	N/A	SJH, Clinical Specimen	1.00E+06 copies/mL	3/3
Human Coronavirus 229E	229E	ATCC, VR-740	2.81E+04 $TCID_{50}$/mL[a]	3/3
SARS-Coronavirus	2003-00592	ZeptoMetrix, NATSARS-ST (NATtrol)	10x dilution of Stock[b]	3/3
SARS-Coronavirus	Frankfurt 1	European Virus Archive, 004N-02005	1.00E+06 copies/mL[c]	3/3
MERS-Coronavirus	Florida/USA-2_Saudi Arabia_2014	ZeptoMetrix, 0810575CFHI	1.00E+05 $TCID_{50}$/mL	3/3
Human Metapneumovirus (hMPV)	IA10-2003	ZeptoMetrix, VPL-030	1.00E+05 $TCID_{50}$/mL	3/3
Rhinovirus	1A	Zeptometrix, 0810012CFN	1.00E+05 $TCID_{50}$/mL	3/3
Enterovirus	US/KY/14-18953	ATCC, VR-1825	1.00E+05 $TCID_{50}$/mL	3/3
	US/IL/14-18952	ATCC, VR-1824	1.00E+05 $TCID_{50}$/mL[d]	3/3
			1.00E+03 $TCID_{50}$/mL	3/3
	2007 Isolate	ZeptoMetrix, 0810237CF	3.42E+03 $TCID_{50}$/mL[a]	3/3
Human respiratory syncytial virus A	A2	ATCC, VR-1540	1.00E+05 $TCID_{50}$/mL	3/3
Human respiratory syncytial virus B	18537	ATCC, VR-1580	1.00E+05 $TCID_{50}$/mL	3/3
Human parainfluenza virus 1	C35	ATCC, VR-94	1.00E+05 $TCID_{50}$/mL	3/3
Human parainfluenza virus 2	Greer	ATCC, VR-92	1.00E+05 $TCID_{50}$/mL	3/3
Human parainfluenza virus 3	C 243	ATCC, VR-93	1.00E+05 $TCID_{50}$/mL	3/3

Organism	Strain	Source	Concentration	SARS-CoV-2 Negativity (#negative/ #tested)
Human parainfluenza virus 4A	Type 4A	Zeptometrix, 0810060CF	1.00E+05 $TCID_{50}$/mL	3/3
Human parainfluenza virus 4B	CH 19503	ATCC, VR-1377	9.98E+04 $TCID_{50}$/mL	3/3
Influenza A H1	A/Brisbane/59/07 H1	ZeptoMetrix, 0810036CF	1.00E+05 $TCID_{50}$/mL	3/3
Influenza A H1N1	A/NY/01/2009	Zeptometrix, 0810109CFN	1.00E+05 $TCID_{50}$/mL	3/3
Influenza A H3N2	A/Victoria/3/75	ATCC, VR-822	1.00E+05 $TCID_{50}$/mL	3/3
Influenza B	B/Florida/04/2006	Zeptometrix, 0810037CF	1.00E+05 $TCID_{50}$/mL	3/3
Adenovirus	species C, type 01	Zeptometrix, 0810050CF	1.00E+05 $TCID_{50}$/mL	3/3
Chlamydia pneumoniae	TWAR strain TW-183	ATCC, VR-2282	1.58E+04 CFU/mL[a]	3/3
Legionella pneumophila	Philadelphia	Zeptometrix, 0801645	1.00E+06 CFU/mL	3/3
Mycoplasma Pneumoniae	M129	Zeptometrix, 0801579	1.00E+06 CCU/mL	3/3
Pooled Nasopharyngeal Swab samples	N/A	Clinical Specimens	N/A	3/3

[a] Highest available titer

[b] No titer information available

[c] Calculated concentration of RNA in the sample pre-extraction

[d] Cross-reaction observed with Influenza A H3. One Enterovirus strain (ATCC PN:VR-1824) generated Influenza A H3 false positive calls when tested at 1.00E+05 $TCID_{50}$/mL. The strain no longer generated false positive calls when tested at 1.00E+03 $TCID_{50}$/mL. Other Enterovirus strains did not cross-react.

Interference

Interference Tested with NxTAG® Respiratory Pathogen Panel + SARS-CoV-2

The potential for non-panel respiratory pathogens (not-detected by the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2) to interfere with the detection of SARS-CoV-2 was assessed by testing pathogens that had ≥80% homology to one of the SARS-CoV-2 primers/probes (*Table 17*). These organisms included *Candida albicans*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and SARS-Coronavirus. No interference with SARS-CoV-2 detection was observed.

Table 17. Microbial Interference with SARS-CoV-2 by Off-Panel Organisms in NxTAG® RPP + SARS-CoV-2

Interfering Organism	Strain	Source	Concentration	SARS-CoV-2 Positivity
Candida albicans	Z006	ZeptoMetrix, 0801504	1.00E+06 CFU/mL	3/3
Mycobacterium tuberculosis	H37Ra	ZeptoMetrix, 0801660	1.00E+06 CFU/mL	3/3
Streptococcus pneumoniae	Zo22, 19F	ZeptoMetrix, 0801439	1.00E+06 CFU/mL	3/3
Streptococcus pyogenes	Z018	ZeptoMetrix, 0801512	1.00E+06 CFU/mL	3/3
SARS-Coronavirus	2003-00592	ZeptoMetrix, NATSARS-ST	10x dilution from stock*	3/3

* No titer information available.

The potential for on-panel competitive inhibition of commonly occurring co-infections involving SARS-CoV-2 was evaluated. Simulated co-infections were prepared in triplicate with SARS-CoV-2 at either a high concentration (1.00E+06 copies/mL) or a low concentration of 3x LoD (1.50E+03 copies/mL) with the competing on-panel target at either a low concentration of 3x LoD or a high concentration (1.00E+05 $TCID_{50}$/mL for viruses, 1.00E+06 copies/mL, or at the highest concentration possible), respectively. No competitive inhibition was observed (*Table 18*).

Table 18. Competitive Inhibition between SARS-CoV-2 and on-panel organisms in NxTAG® RPP + SARS-CoV-2

Target-1	Target-2	Concentration		SARS-CoV-2 Positivity
		Target-1	Target-2	
SARS-CoV-2	Influenza A H1N1 (A/Mexico/4108/09)	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	1.66E+00 $TCID_{50}$/mL	3/3

Target-1	Target-2	Concentration		SARS-CoV-2 Positivity
		Target-1	Target-2	
SARS-CoV-2	Influenza A H3 (A/Texas/71/2007)	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	7.50E-01 $TCID_{50}$/mL	3/3
SARS-CoV-2	Influenza A H3 (Victoria/3/75)	1.50E+03 Copies/mL	1.00E+05 $CEID_{50}$/mL	3/3
		1.00E+06 Copies/mL	4.79E+01 $CEID_{50}$/mL	3/3
SARS-CoV-2	Influenza B (B/Florida/04/2006)	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	1.74E+00 $TCID_{50}$/mL	3/3
SARS-CoV-2	Human Respiratory Syncytial Virus A	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	6.45E+00 $TCID_{50}$/mL	3/3
SARS-CoV-2	Human Coronavirus-NL63	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	1.01E-02 $TCID_{50}$/mL	3/3
SARS-CoV-2	Human Coronavirus-OC43	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	2.15E-01 $TCID_{50}$/mL	3/3
SARS-CoV-2	Human Metapneumovirus (hMPV)	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	4.14E-01 $TCID_{50}$/mL	3/3
SARS-CoV-2	Rhinovirus	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
		1.00E+06 Copies/mL	1.55E+00 $TCID_{50}$/mL	3/3
SARS-CoV-2	Respiratory Syncytial Virus B	1.50E+03 Copies/mL	8.89E+04 $TCID_{50}$/mL*	3/3
SARS-CoV-2	Coronavirus 229E	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
SARS-CoV-2	Coronavirus HKU1	1.50E+03 Copies/mL	1.21E+05 Copies/mL*	3/3
SARS-CoV-2	Enterovirus D68	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
SARS-CoV-2	Adenovirus B	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
SARS-CoV-2	Parainfluenza Virus 1	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
SARS-CoV-2	Parainfluenza Virus 2	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3

Target-1	Target-2	Concentration		SARS-CoV-2 Positivity
		Target-1	Target-2	
SARS-CoV-2	Parainfluenza Virus 3	1.50E+03 Copies/mL	1.00E+05 $TCID_{50}$/mL	3/3
SARS-CoV-2	Parainfluenza Virus 4	1.50E+03 Copies/mL	1.0E+05 $TCID_{50}$/mL	3/3
SARS-CoV-2	Human Bocavirus	1.50E+03 Copies/mL	1.00E+06 Copies/mL	3/3
SARS-CoV-2	*Chlamydophila pneumoniae*	1.50E+03 Copies/mL	1.58E+03 $TCID_{50}$/mL* (4.57E+06 Copies/mL)	3/3
SARS-CoV-2	*Mycoplasma pneumoniae*	1.50E+03 Copies/mL	1.00E+06 CCU/mL	3/3

* The highest concentration possible based on available stock concentration.

Clinical Performance Characteristics by NxTAG® Respiratory Pathogen Panel (2015) Prospective Specimens

The clinical performance of the NxTAG® Respiratory Pathogen Panel was established in two phases using nasopharyngeal swabs (NPS) prospectively collected from pediatric or adult patients suspected of having respiratory tract infection during the 2013/2014 and 2014/2015 flu seasons. In the first phase of the study (2013/2014 flu season) specimens were collected and tested at 2 clinical sites located in the United States from 29-January-2014 to 09-April-2014. Clinical specimens accrued during the second phase of the prospective study (2014/2015 flu season) were collected and tested from 18-January-2015 to 20-March-2015 at 3 clinical sites located in the United States and Canada. The clinical specimen collection sites were chosen based on the types of patients usually referred to and the prevalence of respiratory pathogens to ensure broad coverage of respiratory organisms, patient ages, and geographical regions. A total of 2132 clinical specimens were used for the prospective data set. Of these, 934 were collected during the 2013/2014 Flu season and the remaining 1198 specimens were enrolled during the 2014/2015 Flu season. *Table 19* provides a summary of the general demographic information of the prospectively collected samples that were included in the analysis (combined 2013/2014 and 2014/2015 prospective data set). Demographic information is also presented by clinical site.

Table 19. General Demographic Details for the Combined Prospective Dataset (N=2132)

Gender	Site 1	Site 2	Site 3	Site 4	All Sites
Male	322 (47.7%)	155 (41.3%)	264 (53.0%)	281 (48.1%)	1022 (47.9%)
Female	353 (52.3%)	220 (58.7%)	234 (47.0%)	303 (51.9%)	1110 (52.1%)
Total	675	375	498	584	2132
Age (yrs)					
0 – 1	105 (15.6%)	25 (6.7%)	155 (31.1%)	168 (28.8%)	453 (21.2%)
>1 – 5	66 (9.8%)	22 (5.9%)	92 (18.5%)	70 (12.0%)	250 (11.7%)

Gender	Site 1	Site 2	Site 3	Site 4	All Sites
>5 – 21	87 (12.9%)	73 (19.5%)	101 (20.3%)	92 (15.8%)	353 (16.6%)
>21 – 65	174 (25.8%)	152 (40.5%)	111 (22.3%)	147 (25.2%)	584 (27.4%)
>65	243 (36.0%)	103 (27.5%)	39 (7.8%)	107 (18.3%)	492 (23.1%)
Total	675	375	498	584	2132
Subject Status					
Outpatients	309 (45.8%)	157 (41.9%)	49 (9.8%)	39 (6.7%)	554 (26.0%)
Hospitalized	255 (37.8%)	144 (38.4%)	332 (66.7%)	329 (56.3%)	1060 (49.7%)
Emergency Department	111 (16.4%)	74 (19.7%)	117 (23.5%)	216 (37.0%)	518 (24.3%)
Total	675	375	498	584	2132
Immune Status					
Immunocompromised	116 (17.2%)	89 (23.7%)	0 (0.0%)	59 (10.1%)	264 (12.4%)
Immunocompetent	506 (75.0%)	285 (76.0%)	0 (0.0%)	525 (89.9%)	1316 (61.7%)
Not Determined	53 (7.9%)	1 (0.3%)	498 (100%)	0 (0.0%)	552 (25.9%)
Total	675	375	498	584	2132

All prospective clinical specimens were analyzed by comparator methods for each analyte target at a centralized testing facility, Luminex Molecular Diagnostic, Toronto, ON. An FDA-cleared assay was used as the comparator method for the following targets: Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3 and Human Metapneumovirus. Amplification followed by bi-directional sequencing (using validated primers) directly from extracted clinical specimens using two nucleic acid amplification tests (NAATs) was used as the comparator method for the following targets: Adenovirus, Influenza A (matrix), Influenza A H1, Parainfluenza 4, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Rhinovirus/Enterovirus, Human Bocavirus, *Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae*.

Clinical runs and re-runs using the NxTAG RPP were carried out for clinical specimens following their extraction from either the fresh state (fresh specimens) or thawed state (frozen specimens) using the NucliSENS® easyMAG® method (bioMérieux®, Inc., Durham, NC) according to the manufacturer's instructions. Total extracted nucleic acid material was stored at -70°C prior to testing with NxTAG RPP. Accuracy determinations (Positive and Negative Percent Agreement) were based on the fraction of comparator positive (or negative) results which were also positive (or negative) by the NxTAG RPP. Positive Percent Agreement (PPA) was calculated by dividing the total number of "true positive" NxTAG RPP results (TP) by the sum of the TP and "false negative" (FN) NxTAG RPP results. Negative Percent Agreement (NPA) was calculated by dividing the total number of "true negative" NxTAG RPP results (TN) by the sum of the TN and "false positive" (FP) NxTAG RPP results. A NxTAG RPP result was considered to be a TP or TN result only in the event that it agreed with the comparator method result for the analyte in question. 95% confidence intervals were calculated using the Exact (Clopper-Pearson) method. The clinical performance of the NxTAG RPP assay in the prospective study (N=2132) is summarized for each individual target in *Table 20*.

Out of the 2132 prospective specimens included in the analysis, 2031 (95.3%) generated valid results with NxTAG RPP for all analytes on the first attempt. Invalid results were generated for one or more analyte in 101 specimens tested. All available residual specimens were re-run with NxTAG RPP and generated valid results upon re-test.

Table 20. NxTAG® RPP Clinical Performance (Prospective Sample Set)

Target	PPA		95% CI		NPA	95% CI	# "No Call" by Comparator
Influenza A	259/273*	94.9%	91.5%-97.2%	1822/1859†	98.0%	97.3%-98.6%	0
Influenza A H1	21/21	100.0%	83.9%-100.0%	2091/2111	99.1%	98.5%-99.4%	0
Influenza A H3	203/206‡	98.5%	95.8%-99.7%	1872/1917§	97.7%	96.9%-98.3%	9
Influenza B	87/91‖	95.6%	89.1%-98.8%	2019/2033#	99.3%	98.8%-99.6%	8
Respiratory Syncytial Virus A	73/73	100.0%	95.1%-100.0%	2037/2052**	99.3%	98.8%-99.6%	7
Respiratory Syncytial Virus B	131/133	98.5%	94.7%-99.8%	1978/1990††	99.4%	98.9%-99.7%	9
Coronavirus 229E	21/21	100.0%	83.9%-100.0%	2098/2111	99.4%	98.9%-99.7%	0
Coronavirus OC43	30/31	96.8%	83.3%-99.9%	2092/2101	99.6%	99.2%-99.8%	0
Coronavirus NL63	62/65	95.4%	87.1%-99.0%	2053/2065	99.4%	99.0%-99.7%	2‡‡
Coronavirus HKU1	13/14	92.9%	66.1%-99.8%	2113/2118	99.8%	99.4%-99.9%	0
Human Meta-pneumovirus	135/144§§	93.8%	88.5%-97.1%	1958/1976‖‖	99.1%	98.6%-99.5%	12
Rhinovirus /Enterovirus	286/300 ##	95.3%	92.3%-97.4%	1764/1832***	96.3%	95.3%-97.1%	0
Adenovirus	20/20	100.0%	83.2%-100.0%	2078/2112†††	98.4%	97.8%-98.9%	0
Parainfluenza 1	5/5	100.0%	47.8%-100.0%	2115/2116	99.9%	99.7%-100.0%	11
Parainfluenza 2	1/2‡‡‡	50.0%	1.3%-98.7%	2121/2122	99.9%	99.7%-100.0%	8
Parainfluenza 3	20/21§§§	95.2%	76.2%-99.9%	2086/2103‖‖‖	99.2%	98.7%-99.5%	8
Parainfluenza 4	3/5	60.0%	14.7%-94.7%	2116/2127	99.5%	99.1%-99.7%	0

Target	PPA		95% CI		NPA	95% CI	# "No Call" by Comparator
Human Bocavirus###	27/28	96.4%	81.7%-99.9%	2081/2104	98.9%	98.4%-99.3%	0
Chlamydophila pneumoniae###	0/1	0.0%	0.0%-97.5%	2131/2131	100.0%	99.8%-100.0%	0
Mycoplasma pneumoniae###	7/9	77.8%	40.0%-97.2%	2121/2123	99.9%	99.7%-100.0%	0

* All fourteen (14) Flu A negative specimens that were positive by the reference method (i.e. False Negative) were negative by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

† Twelve (12) Flu A positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

‡ Two (2) Flu A H3 negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

§ Thirty-four (34) Flu A H3 positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

|| Three (3) Flu B negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

Four (4) Flu B positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

** Eight (8) Respiratory Syncytial Virus A positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

†† Three (3) Respiratory Syncytial Virus B positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

‡‡ Two (2) clinical specimens did not have sufficient volume for confirmatory sequencing (QNS).

§§ Six (6) Human Metapneumovirus negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

|||| Two (2) Human Metapneumovirus positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

Eight (8) Rhinovirus/Enterovirus negative specimens that were positive by the reference method (i.e. False Negative) were negative by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

*** Eighteen (18) Rhinovirus/Enterovirus positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

††† Two (2) Adenovirus positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

‡‡‡ The one (1) Parainfluenza 2 negative specimen that was positive by the reference method (i.e. False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

§§§ The one (1) Parainfluenza 3 negative specimen that was positive by the reference method (i.e. False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

||||||| Two (2) Parainfluenza 3 positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP Assay.

Site testing results on discrepant specimens were unavailable for Human Bocavirus, *Chlamydophila pneumonia* and *Mycoplasma pneumoniae.*

NxTAG RPP detected a total of 96 mixed infections in the first phase of the prospective clinical evaluation (2013/2014 flu season). This represents 18.6% of the total number of NxTAG RPP positive specimens (96/517) during that study period. Eighty-four (84/96; 87.5%) were double infections, 8 (8/96; 8.3%) were triple infections, 3 (3/96; 3.1%) were quadruple infections and 1 (1/96; 1.0%) was quintuple infection. The single most common co-infection (9/96; 9.4%) was Human Metapneumovirus with Rhinovirus/Enterovirus. Out of the 96 co-infections, 47 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infections. Distinct co-infection combinations detected by NxTAG RPP during the first phase of the prospective study (2013/2014 flu season) are summarized in *Table 21*.

Table 21. Distinct Co-infections Combination Detected by NxTAG RPP Assay in the Prospective Arm of the Clinical Study (January 2014 to April 2014)

Distinct Co-infection Combination Detected by NxTAG RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4	Target 5			
CoV 229E	Rhino/Entero				1	1	CoV 229E (x1);
CoV 229E	HMPV				3	3	CoV 229E (x3);
CoV HKU1	Adenovirus				1	0	
CoV HKU1	Rhino/Entero				1	0	
CoV HKU1	HMPV				2	0	
CoV NL63	Rhino/Entero				3	2	CoV NL63 (x2); Rhino/Entero (x1);
CoV NL63	HMPV				1	0	

Distinct Co-infection Combination Detected by NxTAG RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4	Target 5			
Rhino/Entero	Adenovirus				4	1	Adenovirus (x1);
Rhino/Entero	HBoV				9	5	Rhino/Entero (x1); HBoV (x5);
Rhino/Entero	*M. Pneu-moniae*				1	0	
HMPV	Adenovirus				3	3	HMPV (x2); Aden-ovirus (x3);
HMPV	Rhino/Entero				9	4	HMPV (x1); Rhino/Entero (x3);
HMPV	HBoV				4	3	HBoV (x3);
Flu A H1	CoV 229E				2	2	Flu A H1 (x2);
Flu A H1	CoV HKU1				2	1	Flu A H1 (x1);
Flu A H1	Rhino/Entero				1	1	Flu A H1; Rhino/Entero (x1);
Flu A H1	HMPV				1	0	
Flu A H1	Flu A H3				1	1	Flu A H3 (x1);
Flu A H3	CoV 229E				1	0	
Flu A H3	CoV NL63				2	0	
Flu A H3	Rhino/Entero				4	2	Flu A H3 (x1); Rhino/Entero (x1);
Flu A H3	RSV A				1	1	Flu A H3 (x1); RSV A (x1);
Flu B	Adenovirus				1	1	Adenovirus (x1);
Flu B	CoV 229E				1	1	CoV 229E (x1);
Flu B	Rhino/Entero				1	1	Rhino/Entero (x1);
Flu B	RSV B				1	0	

Distinct Co-infection Combination Detected by NxTAG RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4	Target 5			
RSV A	Rhino/Entero				3	1	Rhino/Entero (x1);
RSV A	HBoV				1	1	HBoV (x1);
RSV A	PIV 1				1	0	
RSV A	PIV 4				1	1	PIV 4 (x1);
RSV A	RSV B				2	1	RSV A (x1); RSV B (x1);
RSV B	Adenovirus				1	1	Adenovirus (x1);
RSV B	CoV 229E				1	1	CoV 229E (x1);
RSV B	CoV NL63				1	0	
RSV B	CoV OC43				1	0	
RSV B	Rhino/Entero				8	1	Rhino/Entero (x1);
RSV B	HBoV				3	1	HBoV (x1);
Adenovirus	PIV 3	PIV 4			1	1	Adenovirus (x1); PIV 4 (x1);
CoV NL63	Rhino/Entero	HBoV			2	0	
Flu A H1	CoV 229E	CoV NL63			1	1	Flu A H1 (x1); CoV NL63 (x1);
Flu A H3	CoV NL63	Rhino/Entero			1	0	
RSV B	CoV NL63	Rhino/Entero			1	0	
RSV B	CoV NL63	HMPV			2	2	RSV B (x1); CoV NL63 (x1); HMPV (x1);
Flu A H1	PIV 3	PIV 4	*M. Pneumoniae*		1	1	*M. Pneumoniae* (x1);
Flu B	HMPV	PIV 3	PIV 4		1	1	HMPV (x1); PIV 4 (x1);
RSV B	CoV HKU1	Rhino/Entero	Adenovirus		1	0	

Distinct Co-infection Combination Detected by NxTAG RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4	Target 5			
RSV B	CoV NL63	HMPV	Rhino/Entero	HBoV	1	0	
Total Co-infections					96	47	
Double Infections					84	41	
Triple Infections					8	4	
Quadruple Infections					3	2	
Quintuple Infections					1	0	

* A discrepant co-infection or discrepant analyte was defined as one that was detected by NxTAG RPP but not by the reference/comparator methods.

NOTE: The following abbreviations are used for *Table 21*: Flu A = Influenza A; CoV = Coronavirus; Rhino/Entero = Rhinovirus/Enterovirus; HMPV= Human Metapneumovirus; HBoV = Human Bocavirus; M. pneumonia = *Mycoplasma pneumoniae*; RSV= Respiratory Syncytial Virus; PIV = Parainfluenza;

During the second phase of the prospective clinical evaluation (2014/2015 flu season), NxTAG RPP detected a total of 120 mixed infections. This represents 17.3% of the total number of NxTAG RPP positive specimens (120/694) during that study period. 97 (97/120; 80.8%) were double infections, 15 (15/120; 12.5%) were triple infections and 8 (8/120; 6.7%) were quadruple infections. The single most common co-infection (7/120; 5.8%) was Adenovirus with Rhinovirus/Enterovirus. Out of the 120 co-infections, 75 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infections. Distinct co-infection combinations detected by NxTAG RPP during the second phase of the prospective study (2014/2015 flu season) are summarized in *Table 22*.

Table 22. Distinct Co-infections Combination Detected by NxTAG RPP in the Prospective Arm of the Clinical Study (January 2015 to March 2015)

Distinct Co-infection Combination Detected by NxTAG RPP				Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4			
Adenovirus	HBoV			1	0	
Adenovirus	*M. Pneumoniae*			1	1	Adenovirus (x1); *M. Pneumoniae* (x1);
Adenovirus	PIV 3			2	1	PIV 3 (x1);
CoV 229E	CoV NL63			1	0	
CoV 229E	HBoV			1	0	

Distinct Co-infection Combination Detected by NxTAG RPP				Total # co-infections	# discrepant results*	Discrepant Ana-lytes*
Target 1	Target 2	Target 3	Target 4			
CoV 229E	HMPV			1	0	
CoV 229E	PIV 3			1	0	
CoV HKU1	PIV 1			1	1	CoV HKU1 (x1); PIV 1 (x1);
CoV NL63	Adenovirus			1	1	Adenovirus (x1);
CoV NL63	Rhino/Entero			2	1	Rhino/Entero (x1);
CoV NL63	HMPV			3	1	HMPV (x1);
CoV NL63	PIV 4			1	1	CoV NL63 (x1); PIV 4 (x1);
CoV OC43	Adenovirus			1	1	Adenovirus (x1);
CoV OC43	CoV NL63			1	0	
CoV OC43	Rhino/Entero			1	0	
CoV OC43	HBoV			1	1	HBoV (x1);
CoV OC43	HMPV			1	1	HMPV (x1);
Rhino/Entero	Adenovirus			7	7	Rhino/Entero (x7); Adenovirus (x7);
Rhino/Entero	HBoV			3	3	HBoV (x3);
Rhino/Entero	PIV 3			2	2	Rhino/Entero (x1); PIV 3 (x1);
HMPV	Rhino/Entero			4	1	HMPV (x1);
HMPV	PIV 3			1	0	
Flu A (unsub-typeable)	Rhino/Entero			1	1	Flu A (matrix gene) (x1);
Flu A (unsub-typeable)	RSV B			1	1	Flu A (matrix gene) (x1);
Flu A H1	CoV NL63			1	1	CoV NL63 (x1);
Flu A H3	CoV 229E			1	1	Flu A H3(x1);

Distinct Co-infection Combination Detected by NxTAG RPP				Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4			
Flu A H3	CoV HKU1			1	1	CoV HKU1 (x1);
Flu A H3	CoV NL63			2	0	
Flu A H3	CoV OC43			2	0	
Flu A H3	Rhino/Entero			6	3	Rhino/Entero (x3);
Flu A H3	HBoV			2	0	
Flu A H3	HMPV			4	3	HMPV (x3);
Flu A H3	Flu B			1	1	Flu B (x1);
Flu A H3	PIV 1			1	1	Flu A H3(x1);
Flu A H3	PIV 2			1	1	PIV 2 (x1);
Flu A H3	PIV 3			1	1	PIV 3 (x1);
Flu A H3	RSV A			4	3	Flu A H3 (x1); RSV A (x3);
Flu B	Adenovirus			1	1	Adenovirus (x1);
Flu B	Rhino/Entero			4	4	Flu B (x2); Rhino/Entero (x2);
Flu B	HBoV			1	1	Flu B (x1);
Flu B	RSV A			2	2	Flu B (x1); RSV A (x1);
Flu B	RSV B			1	1	RSV B (x1);
PIV 3	HBoV			1	0	
RSV A	CoV OC43			1	1	CoV OC43 (x1);
RSV A	Rhino/Entero			4	1	Rhino/Entero (x1);
RSV A	HBoV			1	0	
RSV A	HMPV			1	1	RSV A (x1);
RSV A	PIV 3			1	0	

Distinct Co-infection Combination Detected by NxTAG RPP				Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4			
RSV B	CoV NL63			1	0	
RSV B	CoV OC43			1	0	
RSV B	Rhino/Entero			5	2	RSV B (x1); Rhino/Entero (x1);
RSV B	HBoV			2	1	HBoV (x1);
RSV B	HMPV			2	0	
RSV B	PIV 4			1	0	
CoV NL63	Rhino/Entero	Adenovirus		1	0	
Rhino/Entero	Adenovirus	PIV 3		1	1	Adenovirus (x1);
HMPV	Adenovirus	HBoV		1	1	Adenovirus (x1); HBoV (x1);
HMPV	Adenovirus	PIV 4		1	1	Adenovirus (x1); PIV 4 (x1);
Flu A (unsubtypeable)	Rhino/Entero	Adenovirus		1	1	Flu A (matrix gene) (x1); Rhino/Entero (x1); Adenovirus (x1);
Flu A H1	RSV B	CoV 229E		1	1	Flu A H1 (x1);
Flu A H3	CoV HKU1	PIV 3		1	1	CoV HKU1 (x1); PIV 3 (x1);
Flu A H3	CoV OC43	HMPV		1	1	HMPV (x1);
Flu A H3	Rhino/Entero	PIV 3		1	1	Flu A H3 (x1); PIV 3 (x1);
Flu A H3	RSV A	Rhino/Entero		1	1	Flu A H3 (x1); RSV A (x1); Rhino/Entero (x1);
Flu B	Adenovirus	PIV 3		1	1	Flu B (x1); Adenovirus (x1); PIV 3 (x1);
Flu B	Rhino/Entero	Adenovirus		1	1	Rhino/Entero (x1); Adenovirus (x1);

Distinct Co-infection Combination Detected by NxTAG RPP				Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4			
RSV A	RSV B	CoV NL63		1	0	
RSV A	RSV B	Rhino/Entero		1	1	RSV A (x1);
RSV B	Rhino/Entero	HBoV		1	0	
CoV 229E	Adenovirus	PIV 3	PIV 4	1	1	CoV 229E (x1); Adenovirus (x1); PIV 4 (x1);
CoV 229E	CoV NL63	Rhino/Entero	Adenovirus	1	1	Rhino/Entero (x1); Adenovirus (x1);
CoV NL63	Rhino/Entero	PIV 3	PIV 4	1	1	CoV NL63 (x1); Rhino/Entero (x1); PIV 4 (x1);
HMPV	Adenovirus	PIV 3	HBoV	1	0	
Flu A H3	CoV NL63	PIV 3	PIV 4	1	1	PIV 4 (x1);
Flu B	CoV 229E	CoV HKU1	Rhino/Entero	1	1	Flu B (x1); CoV 229E (x1); CoV HKU1 (x1); Rhino/Entero (x1);
Flu B	CoV NL63	HMPV	Rhino/Entero	1	1	HMPV (x1);
RSV B	CoV 229E	PIV 3	PIV 4	1	1	CoV 229E (x1); PIV 4 (x1);
Total Co-infections				120	75	
Double Infections				97	56	
Triple Infections				15	12	
Quadruple Infections				8	7	

* A discrepant co-infection or discrepant analyte was defined as one that was detected by NxTAG RPP but not by the reference/comparator methods.

NOTE: the following abbreviations are used for *Table 22*: Flu A = Influenza A; CoV = Coronavirus; Rhino/Entero = Rhinovirus/Enterovirus; HMPV = Human Metapneumovirus; HBoV = Human Bocavirus; *M. pneumonia* = *Mycoplasma pneumoniae*; RSV = Respiratory Syncytial Virus; PIV = Parainfluenza.

Pre-selected Clinical Specimens

Due to low prevalence rates of some of the pathogens in the NxTAG® Respiratory Pathogen Panel, an additional study was conducted and the prospective sample set was supplemented with banked (pre-selected) positive specimens collected at selected sites. In order to minimize bias, pre-selected positive specimens were tested along with negative clinical specimens in a randomized, blinded fashion at 4 testing sites (3 of which were external to the Luminex® site). Clinical run results from pre-selected specimens were analyzed separately from those of the prospective data set and performance of the assay was calculated as Positive Percent Agreement (PPA). All pre-selected and contrived specimens were tested by NxTAG RPP following NucliSENS® easyMAG® extraction from the frozen state. Total extracted nucleic acid material was stored at -70°C prior to testing with NxTAG Respiratory Pathogen Panel. *Table 23* provides a summary of the subject demographic information from the 326 nasopharyngeal swabs that were included in the data analysis of the pre-selected study.

Table 23. General Demographic Details for the Pre-selected Data set (N=326)

Sex	Number of Subjects
Male	179 (54.9%)
Female	147 (45.1%)
Not known	0 (0.0%)
Total	326
Age (yrs)	
0 - 1	75 (23.0%)
>1 - 5	76 (23.3%)
>5 - 21	40 (12.3%)
>21 - 65	83 (25.4%)
>65	52 (16.0%)
Not known	0 (0.0%)
Total	326

A total of 432 clinical specimens were included in the pre-selected study (326 pre-selected positive and 106 negative specimens). Of these, 416 (96.3%) generated valid results with NxTAG RPP for all analytes on the first attempt. Invalid results were generated for one or more analyte in 16 specimens tested. All available residual specimens were re-run with NxTAG Respiratory Pathogen Panel and generated valid results upon re-test.

Table 24 summarizes the Positive agreement between comparator and NxTAG RPP for all pre-selected targets evaluated.

Table 24. Positive Percent Agreement of NxTAG RPP in the Pre-selected Data Set

Target	Positive Agreement		95%CI for Positive Agreement*
	TP / (TP+FN)	Percent	
Adenovirus	30/30	100.0%	88.6% - 100.0%
Influenza A H1	35/35	100.0%	90.1% - 100.0%
Parainfluenza 1	38/38	100.0%	90.8% - 100.0%
Parainfluenza 2	33/33	100.0%	89.6% - 100.0%
Parainfluenza 3	34/34	100.0%	89.8% - 100.0%
Parainfluenza 4	41/42	97.6%	87.7% - 99.6%
Coronavirus 229E	17/17	100.0%	81.6% - 100.0%
Coronavirus OC43	16/16	100.0%	80.6% - 100.0%
Coronavirus NL63	15/15	100.0%	79.6% - 100.0%
Coronavirus HKU1	44/49	89.8%	78.2% - 95.6%
Enterovirus D68	14/14	100.0%	78.5% - 100.0%
Chlamydophila pneumoniae	2/2	100.0%	34.2% - 100.0%
Mycoplasma pneumoniae	4/4	100.0%	51.0% - 100.0%

* Determination of the 95% CI was by the Wilson method.

Confirmatory testing by nucleic acid amplification followed by bi-directional sequencing using the analytically validated primers was conducted on all available specimens tested in the pre-selected arm of the clinical study. More specifically, confirmatory testing was performed for those analytes that were positive by NxTAG RPP but not pre-selected at the banking site in order to determine whether these additional positive calls represented True Positive (TP) or False Positive (FP) clinical results. To the extent possible, sequencing primers targeted genomic regions distinct from those of the kit primers. NxTAG RPP generated 96 additional positive calls (after allowable re-runs) for analytes that were not pre-selected at the banking site. A summary of these additional calls and confirmatory testing results is provided in *Table 25*.

Table 25. Summary of Additional Positive Confirmatory Testing Results – Pre-selected Sample Set

Target	# Additional POS Detected by NxTAG RPP	# Additional POS Confirmed by PCR / bi-directional Sequencing
Influenza A (matrix)	0	N/A

Target	# Additional POS Detected by NxTAG RPP	# Additional POS Confirmed by PCR / bi-directional Sequencing
Influenza A H1	0	N/A
Influenza A H3	9	1
Influenza B	0	N/A
Respiratory Syncytial Virus A	5	2
Respiratory Syncytial Virus B	5	5
Coronavirus 229E	4	0
Coronavirus OC43	2	2
Coronavirus NL63	2	0
Coronavirus HKU1	0	N/A
Human Metapneumovirus	5	4
Rhinovirus/Enterovirus	33	31
Adenovirus	7	2
Parainfluenza 1	1	0
Parainfluenza 2	1	1
Parainfluenza 3	5	0
Parainfluenza 4	1	0
Human Bocavirus	13	8
Chlamydophila pneumoniae	0	N/A
Mycoplasma pneumoniae	3	2
All Targets	96	58

92/96 (95.8%) additional positive results were reported in pre-selected positive specimens while the remaining four (4.2%) were reported in negative clinical specimens. Of these, one was positive for Coronavirus 229E, one for Respiratory Syncytial Virus A, one for Rhinovirus/Enterovirus and one was positive for both Flu A H3 and Coronavirus 229E. The presence of one of the pathogens detected in these 4 specimens was confirmed by bi-directional sequencing (Rhinovirus).

NxTAG Respiratory Pathogen Panel detected a total of 79 mixed infections in the pre-selected arm of the clinical study. This represents 24.2% of the total number of NxTAG Respiratory Pathogen Panel positive specimens (79/326). A total of 65 (65/79; 82.3%) were double infections, 12 (12/79; 15.2%) were triple infections and 2 were quadruple infections (2/79; 2.5%). The single most common co-infection was Adenovirus with Rhinovirus/Enterovirus (7/79; 8.9%). Out of the 79 co-infections, 32 contained one or more analytes that were not confirmed by bi-directional sequencing, i.e. discrepant co-infections.

Contrived Specimens

Due to the limited number of samples positive for atypical bacteria, an additional study was performed using contrived *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* specimens. Contrived samples were prepared by spiking varying concentrations of pathogen into negative clinical specimens. Fifty (50) contrived specimens for each of the two atypical bacteria were prepared for testing based on clinically relevant titers as reported in published scientific literature. The titers selected for each atypical bacteria were as follows: *Chlamydophila pneumoniae* specimens range from 1×10^2 to 2×10^6 copies per mL (Senn et al. 2011; Miyashita et al. 2004); and *Mycoplasma pneumoniae* specimens range from 1×10^2 to 1×10^8 copies/mL (Nilsson et al. 2008, Miyashita et al. 2004; Jiang et al. 2014). A summary of the contrived sample set is provided in *Table 26*. Contrived specimens were tested along with 50 distinct negative clinical specimens in a randomized, blinded fashion at 3 testing sites as per pre-selected specimens.

Table 26. Summary of Contrived Sample Set

Analyte	Strain Information	Source	Spiking Level (copies/mL)	Number of Samples
Negative	N/A	N/A	0	50
Chlamydophila pneumoniae	AR39	ATCC 53592	1.00E+02	5
Chlamydophila pneumoniae	TWAR strain 2023	ATCC VR-1356	1.00E+03	15
Chlamydophila pneumoniae	J-21	ATCC VR-1435	1.00E+03	15
Chlamydophila pneumoniae	AO3	ATCC VR-1452	1.00E+04	5
Chlamydophila pneumoniae	CM-1	ATCC VR-1360	1.00E+05	5
Chlamydophila pneumoniae	TW-183	ATCC VR-2282	1.00E+06	5
Mycoplasma pneumoniae	UTMB-10P	ATCC 49894	1.00E+02	5
Mycoplasma pneumoniae	[Mac] (Type 2)	ATCC 15492	1.00E+03	15
Mycoplasma pneumoniae	M129-B7 (Type 1)	ATCC 29342	1.00E+03	15
Mycoplasma pneumoniae	PI 1428 (Type 1)	ATCC 29085	1.00E+04	5
Mycoplasma pneumoniae	[Bru]	ATCC 15377	1.00E+06	5
Mycoplasma pneumoniae	FH strain of Eaton Agent [NCTC 10119]; (Type 2)	ATCC 15531-TTR	1.00E+08	5

Positive and Negative Agreements of NxTAG® Respiratory Pathogen Panel for all targets evaluated in 100 contrived samples along with 50 negative samples are summarized in *Table 27* and *Table 28*.

Table 27. Positive and Negative Agreement of NxTAG RPP for *Chlamydophila pneumoniae* Contrived Sample Set

Result	Contrived sample		
	Positive	Negative	Total
Positive	50	0	50
Negative	0	50	50
Total	50	50	100
Positive Percent Agreement: 50/50 100.0% (95%CI: 92.9% - 100.0%)			
Negative Percent Agreement: 50/50 100.0% (95%CI: 92.9% - 100.0%)			

Table 28. Positive and Negative Agreement of NxTAG RPP for *Mycoplasma pneumoniae* Contrived Sample Set

Result	Contrived sample		
	Positive	Negative	Total
Positive	50	0	50
Negative	0	50	50
Total	50	50	100
Positive Percent Agreement: 50/50 100.0% (95%CI: 92.9% - 100.0%)			
Negative Percent Agreement: 50/50 100.0% (95%CI: 92.9% - 100.0%)			

Seven (7) additional positive results in 6 contrived samples were reported in the 150 contrived sample set. Of these, two were positive for Flu A, one was positive for both Flu A and Flu A H1, two were positive for Rhinovirus/Enterovirus and one was positive for Coronavirus 229E. The presence of Rhinovirus in one sample was confirmed by bi-directional sequencing.

Non-SARS-CoV-2 *In silico* Cross-Reactivity and Reactivity in NxTAG® Respiratory Pathogen Panel (2015)

Laboratory testing was supplemented with *in silico* data where prediction rules were used to predict reactivity and cross-reactivity of specific Influenza A strains. GenBank sequences for the strains listed in were aligned with all primer sequences in the NxTAG® Respiratory Pathogen Panel assay. Reactivity and cross-reactivity was predicted based on thermodynamic analysis of mismatches between the primers and Influenza A sequences. With the exception of an H5N1 swine strain (A/swine/East Java/UT6010/2007(H5N1)), the strains listed are predicated to react to the Influenza A primers but show no cross-reactivity with the other analyte primers in the NxTAG RPP assay. The H5N1 swine strain is not expected to react.

Table 29. Simulated Reactivity and Cross-Reactivity of NxTAG RPP with Avian, Human and Swine Influenza strains

Host	Subtype	Strain	GenBank ID	Expected Result	Predicted in silico Results	Cross-reactive with others Yes (Y)/No (N)
Avian	H2N2	A/chicken/New York/13828-3/1995(H2N2)	CY014822	Influenza A	Influenza A	N
		A/Japan/305/1957(H2N2)	CY014977	Influenza A	Influenza A	N
		A/Korea/426/1968(H2N2)	CY031596	Influenza A	Influenza A	N
Avian	H3N1	A/blue-winged teal/ALB/452/1983 (H3N1)	CY004635	Influenza A	Influenza A	N
Avian			CY005940	H3	Not Detected	N
Avian	H3N2	A/American black duck-/NorthCarolina/675-075/2004 (H3N2)	GU051136	Influenza A	Influenza A	N
Avian			GU051135	H3	H3	N
Avian	H3N5	A/mallard/Netherlands/2/1999 (H3N5)	CY060264	Influenza A	Influenza A	N
Avian			CY060261	H3	H3	N
Avian	H3N6	A/American black duck-/NewBrunswick/25182/2007(H3N6)	CY047697	Influenza A	Influenza A	N
Avian			CY047696	H3	H3	N
Avian	H3N7	A/northern shoveler/California/HKWF1367/2007 (H3N7)	CY033375	Influenza A	Influenza A	N
Avian			CY033372	H3	H3	N
Avian	H3N8	A/American black duck/Washington/699/1978(H3N8)	GU052300	Influenza A	Influenza A	N
Avian			GU052299	H3	H3	N
Avian	H4N6	A/blue-winged teal/Minnesota/Sg-00043/2007(H4N6)	CY063978	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/Bangladesh/1151-10/2010(H5N1)	HQ156766	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/Hunan/2008	GU182162	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/Hunan/2009	HM172150	Influenza A	Influenza A	N

Host	Subtype	Strain	GenBank ID	Expected Result	Predicted in silico Results	Cross-reactive with others Yes (Y)/No (N)
Avian	H5N1	A/chicken/Primorsky/85/2008	FJ654298	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/Thailand/PC-340/2008	EU620664	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/West Bengal/106181/2008	GU083632	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/West Bengal/193936/2009	GU272009	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/West Bengal/239022/2010	CY061305	Influenza A	Influenza A	N
Avian	H5N1	A/chicken/West Bengal/82613/2008	GU083648	Influenza A	Influenza A	N
Avian	H5N1	A/duck/France/080036/2008	CY046185	Influenza A	Influenza A	N
Avian	H5N1	A/duck/Vietnam/G12/2008	AB593450	Influenza A	Influenza A	N
Avian	H5N1	A/great egret/Hong Kong/807/2008	CY036240	Influenza A	Influenza A	N
Avian	H5N1	A/peregrine falcon/Aomori/7/2011	AB629716	Influenza A	Influenza A	N
Avian	H5N1	A/rook/Rostov-on-Don/26/2007(H5N1)	EU814504	Influenza A	Influenza A	N
Avian	H5N1	A/turkey/VA/505477-18/2007(H5N1)	GU186510	Influenza A	Influenza A	N
Avian	H5N2	A/American black duck-/Illinois/08OS2688/2008	CY079453	Influenza A	Influenza A	N
Avian	H5N2	A/American green-winged teal/California/HKWF609/2007	CY033447	Influenza A	Influenza A	N
Avian	H5N2	A/blue-winged teal/Saskatchewan/22542/2007	CY047705	Influenza A	Influenza A	N
Avian	H5N2	A/Canada goose/New York/475813-2/2007	GQ923358	Influenza A	Influenza A	N
Avian	H5N2	A/chicken/Taiwan/A703-1/2008	AB507267	Influenza A	Influenza A	N

Host	Subtype	Strain	GenBank ID	Expected Result	Predicted in silico Results	Cross-reactive with others Yes (Y)/No (N)
Avian	H5N2	A/duck/France/080032/2008	CY046177	Influenza A	Influenza A	N
Avian	H5N2	A/duck/New York/481172/2007	GQ117202	Influenza A	Influenza A	N
Avian	H5N2	A/duck/Pennsylvania/10218/1984 (H5N2)	AB286120	Influenza A	Influenza A	N
Avian	H5N2	A/gadwall/Altai/1202/2007	CY049759	Influenza A	Influenza A	N
Avian	H5N2	A/mallard/Louisiana/476670-4/2007	GQ923390	Influenza A	Influenza A	N
Avian	H5N2	A/waterfowl/Colorado/476466-2/2007	GQ923374	Influenza A	Influenza A	N
Avian	H5N3	A/duck/Singapore/F119/3/1997 (H5N3)	GU052803	Influenza A	Influenza A	N
Avian	H6N1	A/duck/PA/486/1969(H6N1)	EU743287	Influenza A	Influenza A	N
Avian	H6N2	A/mallard/Czech Republic/15902-17K/2009(H6N2)	HQ244433	Influenza A	Influenza A	N
Avian	H7N2	A/chicken/Hebei/1/2002	AY724263	Influenza A	Influenza A	N
Avian	H7N2	A/chicken/New York/23165-6/2005	CY031077	Influenza A	Influenza A	N
Avian	H7N2	A/chicken/NJ/294508-12/2004	EU743254	Influenza A	Influenza A	N
Avian	H7N2	A/chicken/PA/149092-1/02	AY241609	Influenza A	Influenza A	N
Avian	H7N2	A/mallard/Netherlands/29/2006	CY043833	Influenza A	Influenza A	N
Avian	H7N2	A/mallard/Netherlands/29/2006	CY033226	Influenza A	Influenza A	N
Avian	H7N2	A/muscovy duck/New York/87493-3/2005	CY034791	Influenza A	Influenza A	N
Avian	H7N2	A/northern shoveler/California/JN1447/2007	CY076873	Influenza A	Influenza A	N
Avian	H7N7	A/American green-winged teal/Mississippi/09OS046/2009	CY079309	Influenza A	Influenza A	N

Host	Subtype	Strain	GenBank ID	Expected Result	Predicted in silico Results	Cross-reactive with others Yes (Y)/No (N)
Avian	H7N7	A/chicken/Germany/R28/03	AJ619676	Influenza A	Influenza A	N
Avian	H7N7	A/chicken/Netherlands/1/03	AY340091	Influenza A	Influenza A	N
Avian	H7N7	A/mallard/ California/HKWF1971/2007	CY033383	Influenza A	Influenza A	N
Avian	H7N7	A/mute swan/Hungary/5973/2007	GQ240816	Influenza A	Influenza A	N
Avian	H7N7	A/northern shoveler/ Mississippi/09OS643/2009	CY079413	Influenza A	Influenza A	N
Avian	H7N7	A/mallard/Korea/GH171/2007	FJ959087	Influenza A	Influenza A	N
Avian	H9N2	A/turkey/Wisconsin/1/1966(H9N2)	CY014664	Influenza A	Influenza A	N
Avian	H10N7	A/chicken/Germany/N/1949(H10N7)	GQ176135	Influenza A	Influenza A	N
Avian	H11N9	A/duck/Memphis/546/1974(H11N9)	GQ257441	Influenza A	Influenza A	N
Human	H1N1	A/California/UR06-0393/2007 (H1N1)	CY026540	Influenza A	Influenza A	N
Human	H1N1 2009	A/Aalborg/INS133/2009(H1N1)	CY063607	Influenza A	Influenza A	N
			CY063606	H1	H1	N
Human	H1N2	A/New York/297/2003(H1N2)	CY002665	Influenza A	Influenza A	N
Human			CY002664	H1	H1	N
Human	H2N2	A/Albany/20/1957(H2N2)	CY022014	Influenza A	Influenza A	N
Human	H3N2	A/Boston/38/2008(H3N2)	CY044581	Influenza A	Influenza A	N
Human	H3N2	A/Boston/38/2008(H3N2)	CY044580	H3	H3	N
Human	H5N1	A/Bangladesh/3233/2011	CY088772	Influenza A	Influenza A	N
Human	H5N1	A/Cambodia/R0405050/2007(H5N1)	HQ200572	Influenza A	Influenza A	N
Human	H5N1	A/Cambodia/S1211394/2008	HQ200597	Influenza A	Influenza A	N

Host	Subtype	Strain	GenBank ID	Expected Result	Predicted in silico Results	Cross-reactive with others Yes (Y)/No (N)
Human	H5N1	A/Hong Kong/486/97(H5N1)	AF255368	Influenza A	Influenza A	N
Human	H7N2	A/New York/107/2003(H7N2)	EU587373	Influenza A	Influenza A	N
Human	H7N3	A/Canada/rv504/2004(H7N3)	CY015007	Influenza A	Influenza A	N
Human	H7N7	A/Netherlands/219/03(H7N7)	AY340089	Influenza A	Influenza A	N
Human	H9N2	A/Hong Kong/1073/99(H9N2)	AJ278647	Influenza A	Influenza A	N
Swine	H1N1	A/swine/Wisconsin/1/1971 (H1N1)	CY022414	Influenza A	Influenza A	N
Swine			CY022413	H1	Not Detected	N
Swine	H1N2	A/swine/Hong Kong/NS857/2001 (H1N2)	GQ229350	Influenza A	Influenza A	N
Swine			GQ229348	H1	H1	N
Swine	H1N2	A/swine/Sweden/1021/2009(H1N2)	GQ495135	Influenza A	Influenza A	N
Swine			GQ495132	H1	H3	N
Swine	H3N2	A/Indiana/08/2011 (H3N2)	JN655558	Influenza A	Influenza A	N
Swine			JN638733	H3	H3	N
Swine	H3N2	A/Maine/06/2011 (H3N2)	JN866181	Influenza A	Influenza A	N
Swine			JN866186	H3	H3	N
Swine	H3N2	A/swine/NY/A01104005/2011 (H3N2)	JN940425	Influenza A	Influenza A	N
Swine			JN940422	H3	H3	N
Swine	H5N1	A/swine/East Java/UT6010/2007 (H5N1)	HM440124	Influenza A	Not Detected	N

The predicted NxTAG RPP reactivity results based on *in silico* analysis are shown in *Table 30* to *Table 32* for Adenovirus, Human Poliovirus and Coronavirus SARS strains.

Table 30. Simulated Reactivity of NxTAG RPP with Adenovirus strains

Organism	Species	Serotype	GenBank ID	Expected Results	Predicted *in silico* Results
Adenovirus	A	12	AB330093	Adenovirus	Adenovirus
Adenovirus		18	DQ149610	Adenovirus	Not Detected
Adenovirus	B	16	AB330097	Adenovirus	Adenovirus
Adenovirus		35	AB052912	Adenovirus	Adenovirus
Adenovirus		50	DQ149643	Adenovirus	Adenovirus
Adenovirus	D	9	AB330090	Adenovirus	Adenovirus
Adenovirus	D	10	DQ149615	Adenovirus	Adenovirus
Adenovirus	D	13	DQ149616	Adenovirus	Adenovirus
Adenovirus	D	15	DQ149617	Adenovirus	Adenovirus
Adenovirus	D	17	AB330098	Adenovirus	Adenovirus
Adenovirus	D	19	DQ149618	Adenovirus	Adenovirus
Adenovirus	D	20	DQ149619	Adenovirus	Adenovirus
Adenovirus	D	22	DQ149620	Adenovirus	Adenovirus
Adenovirus	D	23	DQ149621	Adenovirus	Adenovirus
Adenovirus	D	24	DQ149622	Adenovirus	Adenovirus
Adenovirus	D	25	DQ149623	Adenovirus	Adenovirus
Adenovirus	D	26	DQ149624	Adenovirus	Adenovirus
Adenovirus	D	27	DQ149625	Adenovirus	Adenovirus
Adenovirus	D	28	DQ149626	Adenovirus	Adenovirus
Adenovirus	D	29	DQ149627	Adenovirus	Adenovirus
Adenovirus	D	30	DQ149628	Adenovirus	Adenovirus
Adenovirus	D	32	DQ149629	Adenovirus	Adenovirus
Adenovirus	D	33	DQ149630	Adenovirus	Adenovirus

Organism	Species	Serotype	GenBank ID	Expected Results	Predicted *in silico* Results
Adenovirus	D	36	DQ149631	Adenovirus	Adenovirus
Adenovirus	D	37	DQ149632	Adenovirus	Adenovirus
Adenovirus	D	38	DQ149633	Adenovirus	Adenovirus
Adenovirus	D	39	DQ149634	Adenovirus	Adenovirus
Adenovirus	D	42	DQ149635	Adenovirus	Adenovirus
Adenovirus	D	43	DQ149636	Adenovirus	Adenovirus
Adenovirus	D	44	DQ149637	Adenovirus	Adenovirus
Adenovirus	D	45	DQ149638	Adenovirus	Adenovirus
Adenovirus	D	46	DQ149639	Adenovirus	Adenovirus
Adenovirus	D	47	DQ149640	Adenovirus	Adenovirus
Adenovirus	D	48	AB330129	Adenovirus	Adenovirus
Adenovirus	D	49	DQ149641	Adenovirus	Adenovirus
Adenovirus	D	51	DQ149642	Adenovirus	Adenovirus
Adenovirus	D	53	FJ169625	Adenovirus	Adenovirus
Adenovirus	D	40	AB330121	Adenovirus	Adenovirus
Adenovirus	D	52	DQ923122	Adenovirus	Adenovirus

Table 31. Simulated Reactivity of NxTAG RPP with Human Poliovirus strains

Organism	Species	Strain	GenBank ID	Expected Results	Predicted *in silico* Results
Human Poliovirus	1	CHN8264c/GZ /CHN/2004	FJ769385	Rhinovirus/Enterovirus	Rhinovirus/Enterovirus
	2	Complete genome	AY177685	Rhinovirus/Enterovirus	Rhinovirus/Enterovirus
	3	IRA10853	EU684056	Rhinovirus/Enterovirus	Rhinovirus/Enterovirus

Table 32. Simulated Reactivity of NxTAG RPP with Coronavirus SARS strains

Organism	Species	Strain	GenBank ID	Expected Results	Predicted *in silico* Results
Coronavirus	SARS	TOR2	AY274119	Not Detected	Not Detected

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This test has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Bocavirus, *Chlamydophila pneumoniae*, and/or *Mycoplasma pneumoniae*, not for any other viruses or pathogens.