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

IVD For *In Vitro* Diagnostic Use.

MLD-056-KPI-010 Rev A
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NxTAG Respiratory Pathogen Panel + SARS-CoV-2 (IVD)

Σ 96 TESTS

CE

EC REP

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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.
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.		
† 	Conformite Europeenne (EU CE Marking of Conformity). CE conformity marking.	5.1.2* 	Authorized representative in the European Community. Indicates the Authorized representative in the European Community.

* ANSI/AAMI/ISO 15223-1:2021, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

† Council Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDMD) (1998)

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

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 is a qualitative test intended for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria extracted from upper respiratory tract specimens collected from individuals with clinical signs and symptoms of a respiratory tract infection. The organism types and subtypes detected by the test are:

Table 1. Targets Probed by the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 Assay

Viral Target	Bacterial Targets
Influenza A	*Chlamydophila pneumoniae*
Influenza A – H1	*Mycoplasma pneumoniae*
Influenza A – 2009 H1N1	*Legionella pneumophila*
Influenza A – H3	
Influenza B	
Respiratory Syncytial Virus A	
Respiratory Syncytial Virus B	
SARS-CoV-2	
Coronavirus 229E	
Coronavirus OC43	
Coronavirus NL63	
Coronavirus HKU1	
Human Metapneumovirus	
Rhinovirus/Enterovirus	
Adenovirus	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	

Viral Target	Bacterial Targets
Parainfluenza 4	
Human Bocavirus	

The test is indicated as an aid in the detection and identification of viral and bacterial agents causing respiratory tract infections in symptomatic adult and pediatric patients who are either hospitalized, admitted to emergency departments, or who are outpatients with suspected respiratory tract infection.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens not detected by this test, or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other pathogens. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory tract infection.

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 is indicated for use with the Luminex® MAGPIX® Instrument and xPONENT® and SYNCT™ software.

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. 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 occur globally affecting between 5% to 10% of adults and 20% to 30% of children (WHO 2012). In Europe, Influenza is estimated to be responsible for approximately 38,500 deaths annually (Preaud et al. 2014). Influenza viruses are members of the *Orthomyxoviridae* family, and are small enveloped particles with an anti- sense 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. 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 Europe, 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 the cause of significant upper and lower respiratory infections in all age groups. In Europe, children prevalence rates of hMPV range from 1.4% to 24% (Divarathna et al. 2020). 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; Zlateva et al. 2020). 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 ill- ness 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 Europe during 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

The Coronavirus Disease 2019 (COVID-19) pandemic caused by a novel coronavirus, SARS-CoV-2, was first detected in Wuhan City, Hubei Province, China. SARS-CoV-2 has the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption.

Non-novel coronaviruses 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).

After the first outbreak of severe acute respiratory syndrome (SARS) in 2003 (Kahn and McIntosh 2005; Drosten et al. 2003; Kuiken et al. 2003), two additional coronaviruses were discovered, HCoV-NL63, and HCoVHKU1 (Rota et al.2003; Esper et al. 2005; van der Hoek et al. 2004).

While prevalence depends on location, in general coronaviruses 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. 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 virus from 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 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; Lenglet 2012). 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).

Legionella pneumophila

Legionella pneumophila is the main cause of Legionnaires' disease (LD), a systemic infectious disease with pneumonia as the main clinical manifestation (Erdogan et al. 2010; Diederen 2008). Legionnaires' disease, and Pontiac fever (PF), an influenza-like, self-limited illness, are the two most common forms of legionellosis caused by *Legionella* bacteria (Hicks, et al. 2012). The bacterium belongs to the genus *Legionella*, which are small gram-negative, aerobic, non-spore-forming bacilli. More than 50 *Legionella* species have been identified with at least 24 species associated with human pneumonia (Newton et al. 2010; Diederen 2008). LD tends to affect the middle-aged and elderly, people who have impaired respiratory and cardiac function, heavy smokers or immunocompromised individuals (Diederen 2008). Incubation time is generally from 2 to 10 days (Guyard and Low 2011; Diederen 2008) and infection is usually by inhalation of aerosols containing the bacteria (Beaute et al. 2013). Early symptoms include headache, myalgia (muscle pain), asthenia, and anorexia. Legionellosis surveillance data between 2000 to 2009 in the U.S. showed that cases tend to occur in the summer and early fall with 62% of the cases in June to October (Hicks et al. 2012).

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 targets and internal controls within each reaction well.

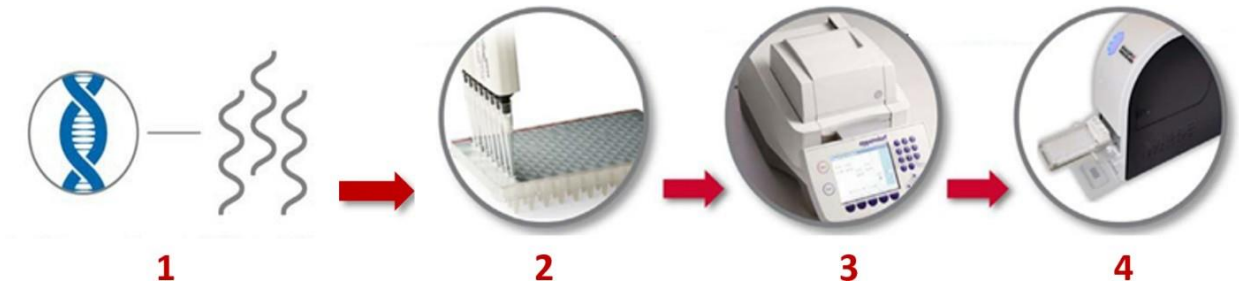
Assay Controls

Good laboratory practice recommends the use of positive and negative controls to ensure functionality of reagents and proper performance of the assay procedure. Positive and negative controls are intended to monitor for substantial failure, contamination, or errors. Results from controls should be examined before reporting results from samples. If a control fails to produce the expected result, all sample results should be examined to determine the validity of the assay run.

NOTE: Controls should be selected and placed on the NxTAG plate in locations that make it possible to determine if the assay plate has been placed on the MAGPIX® instrument in the wrong orientation. For example, replicates of the same control should not be placed in both position 1 (A1) and 96 (H12).

- **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, or 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. Positive controls can be obtained from many commercial suppliers. For the recently added SARS-CoV-2 target Luminex has used inactivated SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Controls from ZeptoMetrix Corporation (Catalog# NATSARS(COV2)-ERC). SARS-CoV-2 run controls were diluted to 5.00E+03 copies/mL in Universal Transport Medium and processed in the same manner as a clinical specimen.

- **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 2. 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 2 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 on USB.

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/Drive Fluid PLUS
- Multichannel pipette or single channel pipette (10 µL to 200 µL)
- Sonicator bath (Ultrasonic Cleaner, Cole-Parmer®, A-08849-00) or equivalent
- 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)
- DNase/RNase-Free Water
- 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 Azenta UK Ltd., 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 Use.
2. For Professional *In Vitro* Diagnostic Use Only. For use by professionals trained to run the NxTAG® RPP + SARS- CoV-2.
3. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
4. 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.
5. 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.
6. 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.
7. Fresh clean gloves must be worn in each area and must be changed before leaving that area.
8. Do not pipette by mouth.
9. For pre-analytical (sample extraction) steps, use the procedure that is provided with the sample extraction system.
10. 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.
11. 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.
12. 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*.
13. Follow your institution's safety procedures for working with chemicals and handling biological samples.
14. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.

15. 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: The total time required to generate results for 24 samples and import to a LIMS system is less than four hours.

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

Collect and 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™ 3C047N), Liquid Amies (ESwab™ 482C), or equivalent (such as MicroTest™ M4™ (R12500), MicroTest™ M4RT™ (R12505), and MicroTest™ M5™ (R12515)). The recommended swab types include nylon flocked swabs, polyester swabs, and rayon swabs.

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 for up to 6 months.

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 for up to 6 months.

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 3. 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 4. 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 5. 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.

Table 6. Thermal Cyclers and Rate Settings

Thermal Cycler	Rate Settings
Eppendorf® Pro S or EP gradient S	75% (~4.5 °C/s)
Bio-Rad® 1000 Series (Fast Module)	5.0 °C/s (with Fast Reaction Module block)
ABI® Veriti	Max (~3.5 °C/s with normal block)

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 setup plate (e.g., skirted plate for Eppendorf® and non-skirted plate for ABI 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[1].lxt2 protocol file. Click **Open**.
5. In the **Imported Protocol File** dialog box, click **OK**. The imported protocol displays 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** 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.

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: NRSC, Assay Version A), skip the following steps:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Assay Management** page.
2. 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_IVD_NRSC_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 exact 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 column to see the results for the individual targets.
1 Positive, 2 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 will 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.

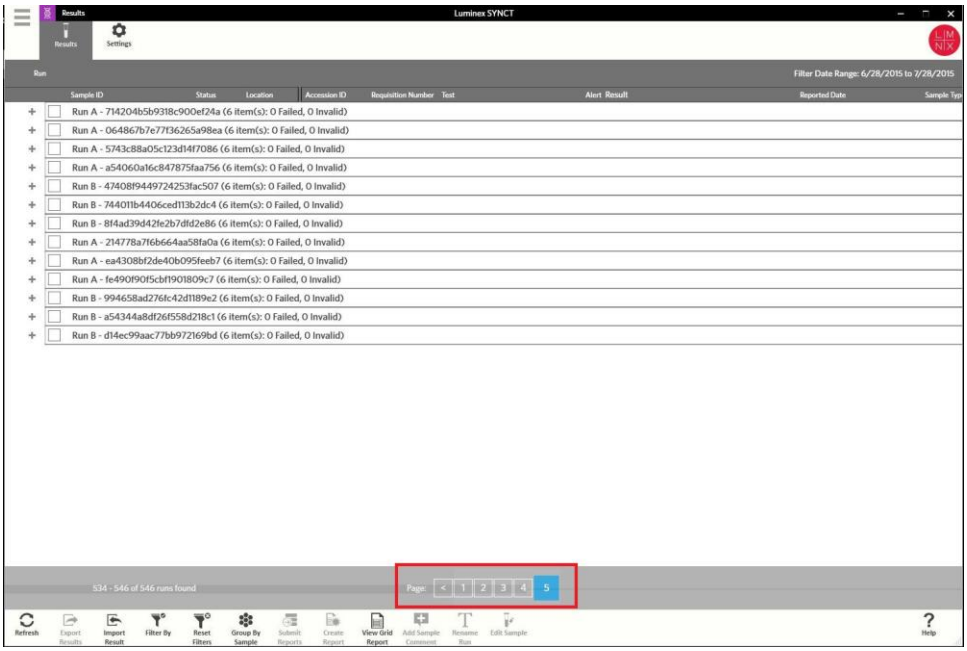
Report Type Definitions

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). Up to 23 targets can be selected for one report.

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 on the left and right arrows to scroll through the pages of results or, if you know what page the results are on, click on 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

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 7. Interpretation of Influenza A Results

Final Result	Influenza A	Influenza A H1	Influenza A 2009 H1N1	H3	Required Follow-Up
Influenza A Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	Positive	Positive	Negative	Negative	None
	Negative[1]	Positive	Negative	Negative	
Influenza A 2009 H1N1	Positive	Negative	Positive	Negative	None
	Negative[1]	Negative	Positive	Negative	
Influenza A H1, Influenza A 2009 H1N1	Positive	Positive	Positive	Negative	None
	Negative[1]	Positive	Positive	Negative	
Influenza A H3	Positive	Negative	Negative	Positive	None
	Negative[1]	Negative	Negative	Positive	
Influenza A H3 and Influenza A H1	Positive	Positive	Negative	Positive	None
	Negative[1]	Positive	Negative	Positive	

Final Result	Influenza A	Influenza A H1	Influenza A 2009 H1N1	H3	Required Follow-Up
Influenza A H3 and Influenza A 2009 H1N1	Positive	Negative	Positive	Positive	
	Negative[1]	Negative	Positive	Positive	
Influenza A (no subtype detected)	Positive	Negative	Negative	Negative	See below

[1] 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.

Influenza A (no subtype detected)

If the Influenza A analyte is positive, but none of the H1 or 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.

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) in the table below.

Table 8. 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 sample.
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.	1. Repeat sample probe height adjustment procedure. Re- run the sample. 2. Ensure the Lyophilized Bead Reagents were fully re-suspended.
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.

Software Result and Messages	Problem	Possible Cause(s)	Recommendation(s)
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 9. 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. This device may not be able to differentiate newly emerging Influenza A subtypes.
2. 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.
3. 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.

4. 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.
5. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
6. 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.
7. 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.
8. A specimen yielding a negative result may contain respiratory pathogens not probed by the assay.
9. 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.
10. The performance of the assay has not been established in individuals who received nasally administered Influenza A vaccine.
11. The performance of this assay was not established in immunocompromised patients.
12. The performance of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 was established using pre-selected, de-identified, nasopharyngeal swabs from 2014 through 2022. The performance for some viruses and subtypes may vary depending on the prevalence and population tested.
13. 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).
14. Performance characteristics for *Chlamydophila pneumoniae*, *Legionella pneumophila,* and *Mycoplasma pneumoniae* were established primarily using contrived specimens. The performance of this test has not been established for screening of blood or blood products.
15. This test cannot rule out infections caused by other viral or bacterial pathogens not present on this panel.
16. Coronavirus 229E may produce a false positive Influenza H1 call.
17. Parainfluenza virus Type 2 may produce a false positive Influenza H3 call.
18. The following potential cross-reactivity is predicted based on *in silico* analysis of primer and probe sequences in the assay against sequences from GenBank nr/nt database available as of May 13, 2020:

    - SARS-CoV-2 oligos are likely to detect some strains of human SARS coronavirus, pangolin coronavirus, and bat coronaviruses.
    - Coronavirus 229E oligos may detect some alpaca respiratory coronavirus and bat 229E-like coronavirus.
    - Coronavirus OC43 oligos may detect some bovine, equine, rabbit. and rodent coronaviruses.
    - Adenovirus oligonucleotides may detect some bacteria that may be of human host (*Cupriavidus pauculus*, *Streptomyces rochei* and *Streptomyces venezuelae*).
    - Some strains of *Pseudomonas putida* may result in a false positive Influenza B result.
    - Some strains of *Pseudomonas parafulva* may result in a false positive *Chlamydia pneumoniae* result.
    - *Legionella pneumophila* oligos may detect several *Acinetobacter* species (*A. baylyi*, *A. beijerinckii*, *A. calcoaceticus*, *chinensis*, *A. equi, A. genomosp*. 9, 11 and 16, A. *guillouiae, A. lwoffii, A. nosocomialis, A. rudis, A. tandoii*, and *A. tjernbergiae*).
    - *Legionella pneumophila* oligos may detect some strains of *Pseudomonas* species (*P. fluorescens, P. koreensis*, and *P. syringae*).

- *Legionella pneumophila* oligos may detect some bacteria that may be of human host (*Moraxellaceae bacterium*, *Myroides* sp., *Neisseria brasiliensis*, *Vagococcus* sp., and *Vitreoscilla* sp.).

19. Other non-2009 H1 Influenza viruses have the potential to give a false positive call for Coronavirus 229E.
20. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
21. This device has been evaluated for use with human specimen material only.
22. The performance of this device has not been evaluated for patients without signs and symptoms of infection.
23. The performance of this device has not been evaluated for monitoring treatment of infection.
24. Assay wells are single-use.

Performance Characteristics

Clinical Performance

The clinical performance of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 was evaluated using leftover, de-identified, and blinded upper respiratory pre-selected clinical specimens. Nasopharyngeal swabs (NPS) were collected in Universal Transport Media (UTM™), various viral transport media (VTM), and liquid amies (ESwab™). Pre-selected clinical specimens were initially selected based on the available molecular assay result, which was then confirmed by reference method testing prior to enrollment. Discordant specimens, where the NxTAG RPP + SARS-CoV-2 assay results differed from the comparator result, were further assessed by PCR followed by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from the NxTAG RPP + SARS-CoV-2 assay. Results from discordant testing analysis were not included in the performance calculations of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA). These results are, however, included as footnotes in the performance evaluation tables. The specimens were collected from symptomatic patients suspected of having respiratory tract infection from twelve geographically diverse clinical sites within the United States and Europe from 2014 through 2022. A total of 606 pre-selected clinical specimens were enrolled in the study, along with 110 contrived clinical specimens prepared with Negative Clinical Matrix (NCM) collected in UTM. *Table 10* provides a summary of the general demographic information (age, gender, and medium) of the pre-selected clinical specimens that were included in the data analysis.

Table 10. General Demographic Details - Pre-selected Dataset (N=606)

Group	Total
Gender	
Female	250
Male	224
Unknown	132
Gender Total	606

Group	Total
Age Group	
0-1	74
>1-5	91
>21-65	82
>5-21	94
>65	49
Unknown	216
Age Total	606
Medium Type	
Liquid Amies	15
MircoTest M4RT	17
MircoTest M4	8
MircoTest M5	2
UTM	564
Medium Type Total	606

Out of the 606 pre-selected clinical specimens included in the analysis, 600 (600/606, 99.0%) generated valid results with NxTAG RPP + SARS-CoV-2 assay on the first attempt. There were 6 specimens (6/606, 0.99%) that were re-tested with NxTAG RPP + SARS-CoV-2 assay because they yielded initial invalid results. All 6 specimens generated valid results upon repeat testing for a final clinical data validity rate of 100%.

Contrived specimens were prepared with Negative Clinical Matrix (NCM), collected in UTM, for *Legionella pneumophila*, Human Bocavirus, Influenza A H1, and Respiratory Syncytial Virus B due to lack of availability of sufficient positive samples. Out of 80 contrived specimens analyzed, all 80 (80/80; 100%) generated valid results with NxTAG RPP + SARS-CoV-2 assay on the first attempt. Contrived specimens for *Legionella pneumophila,* Human Bocavirus, and Influenza A H1 were also prepared for eSwab (liquid amies media) testing. Out of 30 contrived eSwab specimens analyzed, all 30 (30/30; 100%) generated valid results with NxTAG RPP + SARS-CoV-2 assay on the first attempt.

NxTAG RPP + SARS-CoV-2 Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for SARS-CoV-2 and for all other targets are summarized in *Table 11* and *Table 12*.

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

NxTAG® RPP + SARS-CoV-2	Reference Method Result			% Agreement with Reference Method		95% CI
	Positive	Negative	Total			
Positive	120	0	120	PPA	97.6%	93% - 99%
Negative	3[a]	237	240	NPA	100.0%	98%-100%
Total	123	237	360			

[a] One of the three SARS-CoV-2 False Negatives was negative by PCR, followed by bi-directional sequencing.

Table 12. Combined Clinical Performance of NxTAG® RPP + SARS-CoV-2 Assay for all Targets Other Than SARS- CoV-2 in Pre-selected and Contrived Specimens

Target	Sensitivity/PPA			Specificity/NPA			
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI	Total Count
*Legionella pneumophila**	30/30	100%	89% -100%	326/326	100%	99% - 100%	356
Chlamydophila pneumoniae	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Mycoplasma pneumoniae	11/11	100%	74% - 100%	345/345	100%	99% - 100%	356

Target	Sensitivity/PPA			Specificity/NPA			
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI	Total Count
Influenza A H1*	30/30	100%	89% - 100%	326/326	100%	99% - 100%	356
Influenza A 2009 H1N1	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Influenza A H3	9/10[a]	90%	60% - 98%	346/346	100%	99% - 100%	356
Influenza B	10/10	100%	72% - 100%	345/346[f]	99.7%	98% - 100%	356
RSV A	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
RSV B*	22/22	100%	85% - 100%	334/334	100%	99% - 100%	356
Coronavirus 229E	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Coronavirus NL63	9/10[b]	90%	60% - 98%	346/346	100%	99% - 100%	356
Coronavirus OC43	9/9	100%	70% -100%	347/347	100%	99% - 100%	356
Coronavirus HKU1	9/10[c]	90%	60% - 98%	346/346	100%	99% - 100%	356
Human Metapneumovirus	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Adenovirus	18/20[d]	90%	70% - 97%	336/336	100%	99% - 100%	356
Parainfluenza 1	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Parainfluenza 2	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Parainfluenza 3	10/10	100%	72%- 100%	346/346	100%	99% - 100%	356
Parainfluenza 4	10/10	100%	72% - 100%	346/346	100%	99% - 100%	356
Rhinovirus/Enterovirus	12/13[e]	92.3%	67% - 99%	341/343[g]	99.4%	98% - 100%	356
Influenza A	50/50	100%	93%-100%	306/306	100%	99%-100%	356
Human Bocavirus*	31/31	100%	89% -100%	322/325[h]	99.1%	97% - 100%	356

* These targets include 20 (RSV B) or 30 (*Legionella pneumophila,* Influenza A H1, Human Bocavirus) contrived positives each.

[a] The Influenza A H3 False Negative was positive by PCR, followed by bi-directional sequencing.

[b] The Coronavirus NL63 False Negative was negative by PCR, followed by bi-directional sequencing.

[c] The Coronavirus HKU1 False Negative was positive by PCR, followed by bi-directional sequencing.

[d] One of the two Adenovirus False Negatives was negative by PCR, followed by bi-directional sequencing.

[e] The Rhinovirus/Enterovirus False Negative was negative by PCR, followed by bi-directional sequencing.

[f] The Influenza B False Positive was negative by PCR, followed by bi-directional sequencing.

[g] The two Rhinovirus/Enterovirus False Positives were negative by PCR, followed by bi-directional sequencing.

[h] The three Human Bocavirus False Positives were negative by PCR, followed by bi-directional sequencing.

The following tables provide clinical performance evaluation results for pre-selected and contrived specimens for all targets other than SARS-CoV-2 (*Table 13* and *Table 14*).

Table 13. Performance Evaluation of NxTAG® RPP + SARS-CoV-2 Assay for all Targets Other Than SARS-CoV-2 in the Pre-selected Specimens

Organism	Sensitivity/PPA			Specificity/NPA			
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI	Total Count
*Legionella pneumophila**	0/0	N/A	N/A	246/246	100%	98%-100%	246
Chlamydophila pneumoniae	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Mycoplasma pneumoniae	11/11	100%	74% - 100%	235/235	100%	98%-100%	246
Influenza A H1*	0/0	N/A	N/A	246/246	100%	98%-100%	246
Influenza A 2009 H1N1	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Influenza A H3	9/10	90%	60% - 98%	236/236	100%	98%-100%	246
Influenza B	10/10	100%	72% - 100%	235/236	99.6%	98%-100%	246
RSV A	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
RSV B*	2/2	100%	34% - 100%	244/244	100%	98%-100%	246
Coronavirus 229E	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Coronavirus NL63	9/10	90%	60% - 98%	236/236	100%	98%-100%	246
Coronavirus OC43	9/9	100%	70% - 100%	237/237	100%	98%-100%	246
Coronavirus HKU1	9/10	90%	60% - 98%	236/236	100%	98%-100%	246

Organism	Sensitivity/PPA			Specificity/NPA			
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI	Total Count
Human Metapneumovirus	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Adenovirus	18/20	90%	70% - 97%	226/226	100%	98%-100%	246
Parainfluenza 1	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Parainfluenza 2	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Parainfluenza 3	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Parainfluenza 4	10/10	100%	72% - 100%	236/236	100%	98%-100%	246
Rhinovirus/Enterovirus	12/12	100%	76% - 100%	232/234	99.1%	97%-100%	246
Influenza A	20/20	100%	84%-100%	226/226	100%	98%-100%	246
Human Bocavirus*	1/1	100%	21% - 100%	242/245	98.8%	96% - 100%	246

* Additional contrived specimens were added to meet minimum positive sample size requirements. N/A - not applicable for lack of positive specimens.

Table 14. Performance Evaluation of NxTAG® RPP + SARS-CoV-2 Assay for the Contrived Specimens

Target	Sensitivity/PPA			Specificity/NPA			
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI	Total Count
Legionella pneumophila	30/30	100%	89% - 100%	80/80	100%	95% - 100%	110
Influenza A H1	30/30	100%	89% - 100%	80/80	100%	95% - 100%	110
RSV B	20/20	100%	84% - 100%	60/60	100%	94% - 100%	80
Human Bocavirus	30/30	100%	89% - 100%	80/80	100%	95% - 100%	110

Analytical Performance

Limit of Detection (LoD)

The Limit of Detection (LoD) for each of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (RPP + SARS-CoV-2) targets was assessed by analyzing simulated samples made from high-titre pathogen stocks from commercial suppliers or clinical specimens when the target pathogen was not commercially available. NxTAG RPP + SARS-CoV-2 assay is an expanded version of NxTAG Respiratory Pathogen Panel (RPP) assay with an added detection capability of the SARS-CoV-2 target without any modification to the assay components of the NxTAG RPP portion. Therefore, the LoD of targets probed by NxTAG RPP assay on the NxTAG RPP + SARS-CoV-2 assay is expected to remain the same. As a result, the LoD of SARS-CoV-2 was determined and confirmed while the LoD of all other targets also probed by the NxTAG RPP assay were confirmed at the LoD indicated on MLD-051-KPI-002, *Package Insert, NxTAG® Respiratory Panel (*NxTAG RPP*) (IVD), EU, English*. All samples were prepared in negative clinical matrix (NCM). The LoD concentration was considered confirmed if target positivity of ≥ 95% (19/20) was achieved for the respective target when tested at concentrations within 3-fold of the LoD concentration. The summary of the confirmed LoD for each target is listed in *Table 15*.

Table 15. Summary of Confirmed LoD for Targets Detected by NxTAG® RPP + SARS-CoV-2

NxTAG® RPP + SARS-CoV-2 Target	Strain	Concentration	Target Positivity
Influenza A H1 (for matrix)	A/Brisbane/59/07 H1	3.08E+00 $TCID_{50}$/mL	20/20 POS
Influenza A H1 (for subtype)	A/Brisbane/59/07 H1	3.08E+00 $TCID_{50}$/mL	20/20 POS
Influenza A 2009 H1N1 (for matrix)	A/SwineNY/03/2009	5.53E-01 $TCID_{50}$/mL	20/20 POS
Influenza A 2009 H1N1 (for subtype)	A/SwineNY/03/2009	5.53E-01 $TCID_{50}$/mL	20/20 POS
Influenza A H3 (for matrix)	A/Wisconsin/67/05	4.99E-01 $TCID_{50}$/mL*	20/20 POS
Influenza A H3 (for subtype)	A/Wisconsin/67/05	9.36E-02 $TCID_{50}$/mL	20/20 POS
Influenza B	B/Florida/04/2006	5.81E-01 $TCID_{50}$/mL	19/20 POS
Respiratory Syncytial Virus A	A2	2.15E+00 $TCID_{50}$/mL	19/20 POS
Respiratory Syncytial Virus B	18537	1.36E+00 $TCID_{50}$/mL	20/20 POS
SARS-CoV-2	USA-WA1/2020	5.00E+02 Copies/mL	19/20 POS
Coronavirus 229E	229E	1.07E-02 $TCID_{50}$/mL	20/20 POS
Coronavirus OC43	Betacoronavirus 1	7.15E-02 $TCID_{50}$/mL	20/20 POS
Coronavirus NL63	NL63	6.74E-03 $TCID_{50}$/mL*	20/20 POS
Coronavirus HKU1	Clinical Specimen	1.57E+04 Copies/mL	19/20 POS
Human Metapneumovirus	IA10-2003	1.38E-01 $TCID_{50}$/mL	19/20 POS

NxTAG® RPP + SARS-CoV-2 Target	Strain	Concentration	Target Positivity
Rhinovirus	1A	5.18E-01 $TCID_{50}$/mL	20/20 POS
Enterovirus	D68, 2007 Isolate	3.34E+00 $TCID_{50}$/mL	20/20 POS
Adenovirus B	B, Type 14	1.52E-01 $TCID_{50}$/mL	20/20 POS
Adenovirus C	Type 1	3.25E+00 $TCID_{50}$/mL	20/20 POS
Adenovirus E	E, Type 4	1.38E-01 $TCID_{50}$/mL*	20/20 POS
Parainfluenza 1	C35	2.82E+01 $TCID_{50}$/mL	20/20 POS
Parainfluenza 2	Greer	5.36E-01 $TCID_{50}$/mL	20/20 POS
Parainfluenza 3	C 243	3.22E+01 $TCID_{50}$/mL*	20/20 POS
Parainfluenza 4A	Type 4A	5.09E+00 $TCID_{50}$/mL*	20/20 POS
Parainfluenza 4B	CH 19503	6.09E-01 $TCID_{50}$/mL	20/20 POS
Human Bocavirus	Clinical Specimen	3.91E+02 Copies/mL	19/20 POS
Chlamydophila pneumoniae	TWAR strain TW-183	1.29E-01 $TCID_{50}$/mL*	20/20 POS
Mycoplasma pneumoniae	M129	1.42E+02 CCU/mL	20/20 POS
Legionella pneumophila	Philadelphia	3.12E+02 CFU/mL	20/20 POS

*These targets achieved ≥95% (19/20) target positivity when tested at 2-fold the LoD concentration listed in MLD-051- KPI-002, *Package Insert, NxTAG® Respiratory Panel (NxTAG RPP) (IVD), EU, English*.

Matrix Equivalency

A matrix equivalency study was performed to assess the use of negative simulated matrix (NSM; 11 mM NaCl, 0.2 mg/mL mucin, and 1 µg/mL human genomic DNA in UTM) in replacement of negative clinical matrix (NCM) for sample preparation for the proceeding analytical studies on the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay. Two multi-analyte (MA) samples that consist of representative targets of the assay were prepared in NCM and NSM. The MA samples used in this study represent targets of various genome types that are probed by the assay, including single- stranded RNA (ssRNA, positive and negative strand), single-stranded and double-stranded DNA, and bacteria, and could therefore demonstrate the suitability of the two matrices for use in analytical studies. The concentrations of the targets in these MA samples were prepared at the Limit of Detection (LoD). The NxTAG RPP + SARS-CoV-2 assay generated ≥95% target positivity for all targets tested in both NCM and NSM, demonstrating equivalency in the use of NCM and NSM as a sample matrix (*Table 16*). Thus, NSM was used for sample preparation for the proceeding analytical studies for NxTAG RPP + SARS-CoV-2 assay, as applicable.

Table 16. Summary of Target Detectability of NxTAG® RPP + SARS-CoV-2 Assay for Targets in NCM and NSM

Sample Name	Targets	Testing Concentration	Positivity in NCM	Positivity in NSM
NxRPP-CoV-MA1	SARS-CoV-2	5.00E+02 Copies/mL	100% (20/20)	100% (20/20)
	Respiratory Syncytial Virus B	1.36E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
	Human Bocavirus	3.91E+02 Copies/mL	100% (20/20)	95% (19/20)
	Mycoplasma pneumoniae	1.42E+02 CCU/mL	100% (20/20)	100% (20/20)
NxRPP-CoV-MA2	Influenza A-2009 H1N1 (for matrix)	5.53E-01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
	Influenza A-2009 H1N1 (for subtype)	5.53E-01 $TCID_{50}$/mL	100% (20/20)	95% (19/20)
	Coronavirus OC43	7.15E-02 $TCID_{50}$/mL	95% (19/20)	95% (19/20)
	Parainfluenza virus 1	2.82E+01 $TCID_{50}$/mL	100% (20/20)	100% (20/20)
	Adenovirus C	3.25E+00 $TCID_{50}$/mL	100% (20/20)	100% (20/20)

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay was assessed. The study examined 38 reactivity strains in addition to 24 Limit of Detection (LoD) strains, for a total of 62 strains that represent the genetic diversity of the targets probed by the NxTAG RPP + SARS-CoV-2 assay. NxTAG RPP + SARS-CoV-2 assay is an expanded version of NxTAG Respiratory Pathogen Panel (NxTAG RPP) assay with an added detection capability of the SARS-CoV-2 target without any modification to the assay components of the NxTAG RPP portion. Therefore, the detectability of targets probed by NxTAG RPP assay is expected to remain the same for NxTAG RPP + SARS-CoV-2 assay. As a result, a subset of the reactivity strains previously tested with NxTAG RPP assay were prepared and tested at the concentration documented in the NxTAG Respiratory Pathogen Panel package insert (MLD-051-KPI-002) or at three times the LoD (3x LoD) of the corresponding LoD strain.

Three (3) strains of SARS-CoV-2 were tested at 3x LoD, as determined and confirmed during the LoD Study for NxTAG RPP + SARS-CoV-2, along with the LoD strain tested at the LoD concentration.

The summaries of the results for this study, including strain identity and the concentration at which the pathogen were detected at, are shown in *Table 17* to *Table 29*. For the strains that have updated supplier catalogue number, the previous supplier catalogue number of that pathogen listed in the NxTAG Respiratory Pathogen Panel Package Insert is also listed for reference purpose.

Samples from EVAg were obtained as RNA. The RNA was diluted in purified negative clinical matrix to a concentration that represented 1.50E+03 copies/mL in a raw sample.

Four (4) SARS-CoV-2 strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for the SARS-CoV-2 strains are summarized in *Table 17*.

Table 17. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for SARS-CoV-2 Strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK	70034006	4.77E+02	Copies/mL
	USA-WA1/2020	ZeptoMetrix	0810587CFHI	323999	1.50E+03	Copies/mL
	Human 2019-nCoV RNA/BetaCoV/ Germany/BavPat1/2020 RNA	EVAg	026N-03889	N/A	1.50E+03	Copies/mL
	Human 2019-nCoV strain 2019-nCoV/Italy-INMI1 RNA	EVAg	008N-03894	N/A	1.50E+03	Copies/mL

Ten (10) Influenza strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay: 4 of Influenza A H1N1, 3 of Influenza A H3, 1 strain each of Influenza A H5, H7, and H9. The strain information and the concentration detected for Influenza A strains are summarized in *Table 18*. Influenza A H9 (Catalogue Number: FR-1068) was detected at 2-fold of the concentration listed on the NxTAG RPP Package Insert for this strain (1.00E+02 $CEID_{50}$/mL).

Table 18. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Influenza A Strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Matrix or Subtype	Concentration Detected	
Flu A H1N1	A/SwineNY/03/ 2009	ZeptoMetrix	0810109CFN	305985 (sublot 511335)	FluA matrix	5.53E-01	$TCID_{50}$/mL
					H1N1 subtype	5.53E-01	$TCID_{50}$/mL
	A/California/7/ 2009	ZeptoMetrix	0810165CF	308913 (sublot 13984)	FluA matrix	1.66E+00	$TCID_{50}$/mL
					H1N1 subtype	1.66E+00	$TCID_{50}$/mL
	A/Mexico/ 4108/09	ZeptoMetrix	0810166CF	308395 (sublot 13040)	FluA matrix	1.66E+00	$TCID_{50}$/mL
					H1N1 subtype	1.66E+00	$TCID_{50}$/mL
	A/Swine/Canada/ 6294/09	ZeptoMetrix	0810109CFJ	308144 (sublot 13046)	FluA matrix	1.66E+00	$TCID_{50}$/mL
					H1N1 subtype	1.66E+00	$TCID_{50}$/mL

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Matrix or Subtype	Concentration Detected	
Flu A H3	A/Wisconsin/67/05	ZeptoMetrix	0810252CF (PN on RPP PI: 0810138CF)	308394 (sublot 514774)	FluA matrix	2.50E-01	$TCID_{50}$/mL
					H3 Subtype	9.36E-02	$TCID_{50}$/mL
	A/New York/39/2012	IRR	FR-1307	62175007	FluA matrix	7.49E-01	$TCID_{50}$/mL
					H3 Subtype	7.49E-01	$TCID_{50}$/mL
	A/Perth/16/09	ZeptoMetrix	0810251CF (PN on RPP PI: 0810138CF)	307556	FluA matrix	7.49E-01	$TCID_{50}$/mL
					H3 Subtype	7.49E-01	$TCID_{50}$/mL
Flu A H5	A/Egypt/N03072/2010 (H5N1)	IRR	FR-1065	62539792	FluA matrix	1.51E+02	Copies/mL
Flu A H7	A/Turkey/Virginia/4529/2002 (H7N2)	IRR	FR-772	62539793	FluA matrix	1.51E+02	Copies/mL
Flu A H9	A/Hong Kong/33982/2009 (H9N2)	IRR	FR-1068	61220127	FluA matrix	2.00E+02	$CEID_{50}$/mL

Three (3) Influenza B strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for Influenza B strains are summarized in *Table 19*.

Table 19. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Influenza B Strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Flu B	B/Florida/04/2006 (Yamagata)	ZeptoMetrix	0810255CF (PN on RPP PI: 0810037CF)	305764 (sublot 511111)	5.81E-01	$TCID_{50}$/mL
	B/Brisbane/60/08 (Victoria)	ZeptoMetrix	0810254CF	308390 (sublot 513438)	1.74E+00	$TCID_{50}$/mL
	B/Florida/02/06 (Yamagata)	ZeptoMetrix	0810037CF (PN on RPP PI: 0810037CF)	307550 (sublot 511537)	1.74E+00	$TCID_{50}$/mL

Six (6) Respiratory Syncytial Virus (RSV) strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay: 3 of RSV A and 3 of RSV B strains. The strain information and the concentration detected for RSV strains are summarized in *Table 20*. RSV A (Catalogue Number: VR-26) was detected at 2-fold of the concentration listed on the NxTAG RPP Package Insert for this strain (1.65E+03 $TCID_{50}$/mL).

Table 20. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Respiratory Syncytial Virus strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
RSVA	A2	ATCC	VR-1540	58224956 (Reference Lot 4W)	2.15E+00	$TCID_{50}$/mL
	A	ZeptoMetrix	0810040ACF	309017 (sublot 515463)	4.12E+02	$TCID_{50}$/mL
	Long	ATCC	VR-26	58215272 (Reference Lot 22W)	3.30E+03	$TCID_{50}$/mL
RSV B	18357	ATCC	VR-1580	64022963	1.36E+00	$TCID_{50}$/mL
	B WV/14617/85	ATCC	VR-1400	59509416 (Reference Lot 7W)	4.07E+00	$TCID_{50}$/mL
	CH93-18(18)	ZeptoMetrix	0810040CF	308131 (sublot 513226)	6.51E+01	$TCID_{50}$/mL

Ten (10) Parainfluenza Virus (PIV) strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay: 2 of PIV1, 2 of PIV2, 2 of PIV3, 2 of PIV4A, and 2 of PIV4B strains. The strain information and the concentration detected for PIV strains are summarized in *Table 21*.

Table 21. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Parainfluenza Virus Strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
PIV1	C35	ATCC	VR-94	58834906	2.82E+01	$TCID_{50}$/mL
	Type 1	ZeptoMetrix	0810014CF	306018	8.46E+01	$TCID_{50}$/mL
PIV2	Greer	ATCC	VR-92	58159787 (Reference Lot 20W)	5.36E-01	$TCID_{50}$/mL
	Type 2	ZeptoMetrix	0810015CF	309210 (sublot 514876)	1.03E+02	$TCID_{50}$/mL

Organism	Strain	Source	Supplier Catalogue Number	Lot Number		Concentration Detected
PIV3	C 243	ATCC	VR-93	59380357	1.61E+01	$TCID_{50}$/mL
	Type 3	ZeptoMetrix	0810016CF	307006 (sublot 512805)	4.83E+01	$TCID_{50}$/mL
PIV4A	Type 4A	ZeptoMetrix	0810060CF	319729 (sublot 532206)	2.54E+00	$TCID_{50}$/mL
	M-25	ATCC	VR-1378	58486646 (Reference Lot 7W)	7.63E+00	$TCID_{50}$/mL
PIV4B	CH 19503	ATCC	VR-1377	61430657	6.09E-01	$TCID_{50}$/mL
	Type 4B	ZeptoMetrix	0810060BCF	308025	7.31E+00	$TCID_{50}$/mL

Eight (8) Coronavirus strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay: 2 of coronavirus 229E, 2 of coronavirus NL63, 2 of coronavirus OC43, and 2 of coronavirus HKU1 strains. The strain information and the concentration detected for coronavirus strains are summarized in *Table 22*. Coronavirus 229E (Catalogue Number: 0810229CF) was detected at 6-fold of the concentration listed on the NxTAG RPP Package Insert for this strain (5.15E- 01 $TCID_{50}$/mL). Coronavirus OC43 (Catalogue Number: 0810024CF) was detected at 4-fold of the concentration listed on the NxTAG RPP Package Insert for this strain (2.15E-01 $TCID_{50}$/mL).

Table 22. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Coronavirus strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Coronavirus	229E	ATCC	VR-740	58505270	1.07E-02	$TCID_{50}$/mL
	229E	ZeptoMetrix	0810229CF	307701 (sublot 514158)	3.09E+00	$TCID_{50}$/mL
	NL63	ZeptoMetrix	0810228CF	308994 (sublot 515584)	3.37E-03	$TCID_{50}$/mL
	NL63	SJH	50608	N/A	1.01E-02	$TCID_{50}$/mL
	OC43	ATCC	VR-1558	62246951	7.15E-02	$TCID_{50}$/mL
	OC43	ZeptoMetrix	0810024CF	307008 (sublot 512656)	8.60E-01	$TCID_{50}$/mL
	HKU1, Genotype B	Clinical Sample	LMD-05	HKU1-5	1.57E+04	Copies/mL
	HKU1, Genotype A	Clinical Sample	LMD-06	N/A	4.71E+04	Copies/mL

Four (4) Human Metapneumovirus (hMPV) strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for hMPV strains are summarized in *Table 23*.

Table 23. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Human Metapneumovirus strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
hMPV	Subtype A1, IA10-2003, hMPV-16	ZeptoMetrix	VPL-030	305069	1.38E-01	$TCID_{50}$/mL
	Subtype A2, DHI 26583	SJH 030209	DHI 26583	30209	4.15E-01	$TCID_{50}$/mL
	Subtype B1, Peru2- 2002, hMPV-3	ZeptoMetrix	0810156CF	308423	1.77E+01	$TCID_{50}$/mL
	Subtype B2, Peru1- 2002, hMPV-4	ZeptoMetrix	0810157CF (PN on RPP PI: VPL-030)	305227	4.15E-01	$TCID_{50}$/mL

Two (2) Rhinovirus strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for rhinovirus strains are summarized in *Table 24*.

Table 24. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Rhinovirus strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Rhinovirus	Species A, Type 1A	ZeptoMetrix	0810012CFN	305067	5.18E-01	$TCID_{50}$/mL
	Species B, Type 42, Strain 56822	ATCC	VR-338	215603 (Reference Lot 1 WET)	1.55E+00	$TCID_{50}$/mL

Four (4) Enterovirus strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for enterovirus strains are summarized in *Table 25*.

Table 25. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Enterovirus strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Enterovirus	Type D68, strain 2007 isolate	ZeptoMetrix	0810237CF	313095 (sub-lot 518720)	3.34E+00	$TCID_{50}$/mL
	Species A, Type 71, strain H	ATCC	VR-1432	59967091	1.00E+01	$TCID_{50}$/mL
	Species B, Human Echovirus 13, Del Carmen NIAID V-046-001- 010	ATCC	VR-1054	216233	1.00E+01	$TCID_{50}$/mL
	Species C, Human coxsackievirus A24, strain DN-19	ATCC	VR-1662	58528678	1.00E+01	$TCID_{50}$/mL

Five (5) Adenovirus strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay: 1 of Adenovirus A, 1 of Adenovirus B, 1 of Adenovirus C, 1 of Adenovirus D, and 1 of Adenovirus E. The strain information and the concentration detected for adenovirus strains are summarized in *Table 26*.

Table 26. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for Adenovirus strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Adenovirus	Species B, Type 14	ZeptoMetrix	0810108CF	309028	1.52E-01	$TCID_{50}$/mL
	Species C, Type 1	ZeptoMetrix	0810050CF	305544	3.25E+00	$TCID_{50}$/mL
	Species E, Type 4	ZeptoMetrix	0810070CF	305205 (sublot 509205)	6.91E-02	$TCID_{50}$/mL
	Species A, Type 12, Strain Huie	ATCC	VR-863	70027684	2.63E+02	$TCID_{50}$/mL
	Species D, Type 30, Strain BP-7	ATCC	VR-273	215330	2.07E-01	$TCID_{50}$/mL

Two (2) *Chlamydophila pneumoniae* strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for *C. pneumoniae* strains are summarized in *Table 27*.

Table 27. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for *Chlamydophila pneumoniae* strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Chlamydophila pneumoniae	TW-183	ATCC	VR-2282	7565358 (ref lot 7W)	6.43E-02	$TCID_{50}$/mL
	TWAR 2023	ATCC	VR-1356	5040952	1.93E-01	$TCID_{50}$/mL

Two (2) *Mycoplasma pneumoniae* strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for *M. pneumoniae* strains are summarized in *Table 28*.

Table 28. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for *Mycoplasma pneumoniae* strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Mycoplasma pneumoniae	M129	ZeptoMetrix	801579	324216	1.42E+02	CCU/mL
	[M52]	ATCC	15293	59561144	2.11E+03	Copies/mL

Two (2) *Legionella pneumophila* strains were tested for reactivity on the NxTAG RPP + SARS-CoV-2 assay. The strain information and the concentration detected for *L. pneumophila* strains are summarized in *Table 29*.

Table 29. Summary of NxTAG® RPP + SARS-CoV-2 Assay Analytical Reactivity for *Legionella pneumophila* strains

Organism	Strain	Source	Supplier Catalogue Number	Lot Number	Concentration Detected	
Legionella pneumophila	Philadelphia	ZeptoMetrix	801645	320600	3.12E+02	CFU/mL
	Knoxville-1 [NCTC 11286]	ATCC	33153	57835132	5.44E+02	Copies/mL

Analytical Specificity (Cross Reactivity, Microbial Interference, and Competitive Inhibition)

The NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay was assessed for potential cross-reactivity, microbial interference, and co-infection (competitive inhibition) by common respiratory pathogens. A total of 34 organisms (12 off-panel organisms and 22 on-panel organisms, 38 strains total) were assessed for potential cross-reactivity. Five (5) organisms were assessed for potential microbial interference. Twelve (12) pairs of organisms were tested for potential competitive inhibition.

NxTAG RPP + SARS-CoV-2 assay is an expanded version of NxTAG RPP assay with an added detection capability of the SARS-CoV-2 target without any modification to the assay components of the NxTAG RPP portion. Therefore, the analytical specificity of the NxTAG RPP + SARS-CoV-2 assay is expected to remain the same as NxTAG RPP. Therefore, for this study, a subset of the cross-reactivity strains previously tested with NxTAG RPP assay were prepared and tested. In addition, the potential of microbial interference against the SARS-CoV-2 target (ZeptoMetrix PN: 0810587CFHI) and the potential of competitive inhibition of the detection of other on-panel targets by SARS-CoV-2 (ZeptoMetrix PN: 0810587CFHI or ATCC PN: VR-1986HK) were assessed on the NxTAG RPP + SARS-CoV-2 assay.

Cross-Reactivity

Potential cross-reactivity by common respiratory pathogen on the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay was assessed by testing 12 off-panel organisms and 22 on-panel organisms for a total of 38 strains. Cross reactivity was evaluated using simulated specimens by spiking cultured organisms into negative clinical matrix (NCM) or negative simulated matrix (NSM). Viral and bacterial targets were prepared at 1.0E+05 $TCID_{50}$/mL, 1.0E+05 $CEID_{50}$/mL, 1.0E+06 CFU/mL, 1.0E+06 CCU/mL, or 1.0E+06 Copies/mL, or at the highest concentration possible.

Samples from EVAg were obtained as RNA. The RNA was diluted in purified negative clinical matrix to a concentration that represented 1.00E+06 Copies/mL in a raw sample.

All potential cross-reacting off-panel organisms tested on NxTAG RPP + SARS-CoV-2 assay generated negative results for all targets and thus, they do not cross react with the assay (*Table 30*).

Table 30. NxTAG® RPP + SARS-CoV-2 Assay Results for Potential Cross-Reacting Off-panel Organisms

Organism	Supplier	Supplier Catalogue Number	Concentration Detected		Cross-Reactive Yes (Y)/No (N)
Bordetella pertussis	ZeptoMetrix	0801459	1.00E+06	CFU/mL	N
Candida albicans	ZeptoMetrix	0801504	1.00E+06	CFU/mL	N
Haemophilus influenzae	ZeptoMetrix	0801680	1.00E+06	CFU/mL	N
Mycobacterium tuberculosis	ZeptoMetrix	0801660	1.00E+06	CFU/mL	N
Pneumocystis jirovecii (PJP)	ZeptoMetrix	0801698	1.00E+06	CFU/mL	N
Pseudomonas aeruginosa	ZeptoMetrix	0801519	1.00E+06	CFU/mL	N
Staphylococcus epidermidis (MRSE)	ZeptoMetrix	0801651	1.00E+06	CFU/mL	N
Streptococcus pneumoniae	ZeptoMetrix	0801439	1.00E+06	CFU/mL	N
Streptococcus pyogenes	ZeptoMetrix	0801512	1.00E+06	CFU/mL	N
Streptococcus salivarius	ZeptoMetrix	0801896	1.00E+06	CFU/mL	N
SARS-coronavirus	ZeptoMetrix	NATSARS-ST (NATtrol)	10x dilution of Stock*		N
SARS-CoV-1	EVAg	004N-02005	1.00E+06	Copies/mL	N
MERS-coronavirus	ZeptoMetrix	0810575CFHI	1.00E+05	$TCID_{50}$/mL	N

*This is NATtrol™ Coronavirus-SARS from ZeptoMetrix, and no concentration was provided on the CoA. Thus, this was the highest concentration possible based on the available stock.

One on-panel organism tested on NxTAG RPP + SARS-CoV-2 assay generated unexpected false positive calls. Enterovirus (ATCC PN: VR-1824) generated Influenza A H3 false positive calls when tested at 1.00E+05 $TCID_{50}$/mL. The Enterovirus (ATCC PN: VR-1824) strain no longer generated false positive calls when tested at 1.00E+03 $TCID_{50}$/mL. All other potential cross-reacting on-panel organisms tested on NxTAG RPP + SARS-CoV-2 assay generated negative calls for all targets except for their respective target calls. Thus, these on-panel organisms do not cross react with the assay (*Table 31*).

Table 31. NxTAG® RPP + SARS-CoV-2 Assay Results for Potential Cross-reacting On-panel Organisms

Organism	Supplier	Supplier Catalogue Number	Concentration Tested		Cross-Reactive Yes (Y)/No (N)
Human Coronavirus-OC43	ATCC	VR-1558	1.00E+05	$TCID_{50}$/mL	N
Human Coronavirus-NL63	ZeptoMetrix	0810228CF	1.00E+05	$TCID_{50}$/mL	N
Human Coronavirus-HKU1	SJH	Clinical specimen	1.00E+06	Copies/mL	N
Human coronavirus-229E	ATCC	VR-740	2.81E+04*	$TCID_{50}$/mL	N
Human Metapneumovirus (hMPV)	ZeptoMetrix	VPL-030	1.00E+05	$TCID_{50}$/mL	N
Rhinovirus	ZeptoMetrix	0810012CFN	1.00E+05	$TCID_{50}$/mL	N
Enterovirus	ATCC	VR-1825	1.00E+05	$TCID_{50}$/mL	N
	ATCC	VR-1824	1.00E+05	$TCID_{50}$/mL	Y
			1.00E+03	$TCID_{50}$/mL	N
	ZeptoMetrix	0810237CF	3.42E+03	$TCID_{50}$/mL	N
Human respiratory syncytial virus A	ATCC	VR-1540	1.00E+05	$TCID_{50}$/mL	N
Human respiratory syncytial virus B	ATCC	VR-1580	1.00E+05	$TCID_{50}$/mL	N
Human parainfluenza virus 1	ATCC	VR-94	1.00E+05	$TCID_{50}$/mL	N
Human parainfluenza virus 2	ATCC	VR-92	1.00E+05	$TCID_{50}$/mL	N
Human parainfluenza virus 3	ATCC	VR-93	1.00E+05	$TCID_{50}$/mL	N
Human parainfluenza virus 4A	ZeptoMetrix	0810060CF	1.00E+05	$TCID_{50}$/mL	N
Human parainfluenza virus 4B	ATCC	VR-1377	9.98E+04*	$TCID_{50}$/mL	N
Influenza A H1	ZeptoMetrix	0810036CF	1.00E+05	$TCID_{50}$/mL	N
Influenza A H1N1 (A/Swine NY/01/2009)	ZeptoMetrix	0810109CFN (LN: 308135)	1.00E+05	$TCID_{50}$/mL	N
Influenza A H1N1 (A/Swine NY/03/2009)	ZeptoMetrix	0810109CFN (LN: 305985)	1.00E+05	$TCID_{50}$/mL	N

Organism	Supplier	Supplier Catalogue Number	Concentration Tested		Cross-Reactive Yes (Y)/No (N)
Influenza A H3N2	ATCC	VR-822	1.00E+05	$CEID_{50}$/mL	N
Influenza B	ZeptoMetrix	0810037CF	1.00E+05	$TCID_{50}$/mL	N
Adenovirus	ZeptoMetrix	0810050CF	1.00E+05	$TCID_{50}$/mL	N
Chlamydophila pneumoniae	ATCC	VR-2282	1.58E+04*	$TCID_{50}$/mL	N
Legionella pneumophila	ZeptoMetrix	801645	1.00E+06	CFU/mL	N
Mycoplasma pneumoniae	ZeptoMetrix	801579	1.00E+06	CCU/mL	N

* The highest concentration possible based on available stock concentration.

Microbial Interference

Five (5) of the potential cross-reacting off-panel organisms were also tested for potential microbial interference against SARS-CoV-2 target on NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay. These have greater than or equal to 80% homology to one of the SARS-CoV-2 primers/probes. These 5 organisms were spiked into negative clinical matrix (NCM) containing SARS-CoV-2 target at a concentration of 3x Limit of Detection (LoD) and tested in triplicates on the assay. All samples generated 100% (3/3) SARS-CoV-2 positive calls, while 0% positivity was generated for all other targets. Thus, these organisms present in high concentrations are considered non-interfering with the detection of SARS- CoV-2 present in low concentration (*Table 32*).

Table 32. NxTAG® RPP + SARS-CoV-2 Assay Results for Potential Microbial Interfering Organisms

#	Target-1	Target-2	Concentration			Positivity of SARS-CoV-2 Target
			Target-1	Target-2		
1	SARS-CoV-2	*Candida albicans*	1.50E+03 Copies/mL	1.00E+06	CFU/mL	100% (3/3)
2		*Mycobacterium tuberculosis*		1.00E+06	CFU/mL	100% (3/3)
3		SARS-coronavirus		10x dilution of Stock*		100% (3/3)
4		Streptococcus pneumoniae		1.00E+06	CFU/mL	100% (3/3)
5		Streptococcus pyogenes		1.00E+06	CFU/mL	100% (3/3)

* This is NATtrol™ Coronavirus-SARS from ZeptoMetrix, no concentration provided on the CoA. Thus, this was the highest concentration possible based on the available stock.

Competitive Inhibition (Co-Infection)

The competitive inhibition of NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 assay was assessed by testing 12 pairs of on-panel targets. Each pair was tested twice, once with Target-1 at low concentration and Target-2 at high concentration, and second time with Target-1 at high concentration and Target-2 at low concentration. The targets were prepared at 3x LoD for the low concentration while the high concentrations tested were ≥ 1.0E+06 Copies/mL, ≥1.0E+05 $TCID_{50}$/mL, ≥1.0E+05 $CEID_{50}$/mL, or highest available concentration. All samples were tested in triplicates. Across all the combination tested, both organisms present in high and low concentrations were detected (*Table 33*). Thus, these organisms present in high concentrations are considered non-interfering with the detection of other on-panel targets present in low concentration (*Table 33*).

Table 33. NxTAG® RPP + SARS-CoV-2 Assay Results for Potential Competitive Inhibiting Organisms

#	Target-1	Target-2	Concentration				Positivity of Target-1	Positivity of Target-2
			Target-1		Target-2			
1	SARS-CoV-2	Influenza A H3 (Victoria/3/75)	1.50E+03	Copies/mL	1.00E+05	$CEID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	4.79E+01	$CEID_{50}$/mL	100% (3/3)	100% (3/3)
2	SARS-CoV-2	Human respiratory syncytial virus A	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	6.45E+00	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
3	SARS-CoV-2	Human Coronavirus-NL63	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	1.01E-02	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
4	SARS-CoV-2	Human Coronavirus-OC43	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	2.15E-01	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
5	SARS-CoV-2	Human Metapneumovirus (hMPV)	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	4.14E-01	$TCID_{50}$/mL	100% (3/3)	100% (3/3)

#	Target-1	Target-2	Concentration				Positivity of Target-1	Positivity of Target-2
			Target-1		Target-2			
6	SARS-CoV-2	Rhinovirus	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	1.55E+00	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
7	SARS-CoV-2	Influenza A H1N1 (A/Mexico/4108/09)	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	1.66E+00	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
8	SARS-CoV-2	Influenza A H3 (A/Texas/71/2007)	1.50E+03	Copies/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+06	Copies/mL	7.50E-01	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
9	Human respiratory syncytial virus B	Rhinovirus	4.08E+00	$TCID_{50}$/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+05	$TCID_{50}$/mL	1.55E+00	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
10	Human Metapneumovirus (hMPV)	Rhinovirus	4.14E-01	$TCID_{50}$/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+05	$TCID_{50}$/mL	1.55E+00	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
11	Enterovirus	Adenovirus B	1.00E+01	$TCID_{50}$/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+05	$TCID_{50}$/mL	4.56E-01	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
12	Human Coronavirus-NL63	Influenza A H3 (A/Wisconsin/67/05)	1.01E-02	$TCID_{50}$/mL	1.00E+05	$TCID_{50}$/mL	100% (3/3)	100% (3/3)
			1.00E+05	$TCID_{50}$/mL	2.81E-01	$TCID_{50}$/mL	100% (3/3)	100% (3/3)

Interfering Substances

The accuracy of NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay in the presence of potential interfering substances (IFS) was assessed. Eighteen (18) non-microbial substances commonly found in respiratory specimens were tested on the assay alone and in the presence of two (2) multi-analyte (MA) samples, each consisting of 4 representative targets of the assay prepared at 3x Limit of Detection (LoD) (For MA composition, refer to *Table 16*). Based on the results, none of the substances tested (*Table 34*) showed interference with the NxTAG RPP + SARS-CoV-2 assay with the exception of FluMist®. Similarly to NxTAG RPP assay, NxTAG RPP + SARS-CoV-2 assay detected and made positive calls for the attenuated viruses present in the FluMist vaccine (Influenza A, Influenza A 2009 H1N1, Influenza A H3, and Influenza B). This is expected and is a limitation of the assay when FluMist is present in the sample. Positive influenza results obtained in a patient who received FluMist prior to sample collection may be due to detection of the vaccine virus and may mask a true positive result due to infection by one or more of these analytes. All remaining substances tested alone generated 0% positivity for any targets while the substances tested in the presence of MA samples generated 100% positivity of targets present in the MA samples were generated (*Table 34*).

Table 34. Summary of the Evaluation Of Potential Interfering Substances for NxTAG® RPP + SARS-CoV-2 Assay

IFS	Substance	Concentration Tested	Positivity for Expected Targets		Positivity for Other Targets
			MA1	MA2	NSM
IFS-01	Blood	5 %v/v	100% (3/3)	100% (3/3)	0% (0/3)
IFS-02	Human Genomic DNA	2.0E+01 ng/µL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-03	Mucin	100 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-04	Phenylephrine	0.03 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-05	Beclomethasone dipropionate	8.4 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-06	Dexamethasone	12 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-07	Flunisolide	5 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-08	Triamcinolone acetonide	22 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-09	Budesonide	6.30E-03 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-10	Mometasone furoate	4.50E-04 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-11	Fluticasone	1.26E-03 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-12	Drixoral® (Oxymetazoline)	10 % v/v	100% (3/3)	100% (3/3)	0% (0/3)
IFS-13	ZICAM® (Galphimia glauca, Histaminum hydrochloricum)	1% v/v	100% (3/3)	100% (3/3)	0% (0/3)
IFS-14	Salinex (Sodium chloride)	1% v/v	100% (3/3)	100% (3/3)	0% (0/3)

IFS	Substance	Concentration Tested	Positivity for Expected Targets		Positivity for Other Targets
			MA1	MA2	NSM
IFS-15	Mupirocin	1.5 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-16	Tobramycin	33 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-17	Zanamivir	100 µg/mL	100% (3/3)	100% (3/3)	0% (0/3)
IFS-18	FluMist®	0.5% v/v	100% (3/3)	100% (3/3)	100% (3/3)*

* 3/3 replicates for FluMist (when tested both alone and in the multi-analyte samples) generated positive calls for the viral strains in FluMist: Influenza A H1N1, Influenza A H3N2, and Influenza B.

Site-to-Site Reproducibility

Site-to-site reproducibility testing was performed to assess the total variability of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay across operators, study sites, testing days, and instruments. One (1) operator at each of 3 sites tested a blinded set of 5-member reproducibility panel in 4 replicates on 5 non-consecutive days, for a total of 15 runs (1 operator x 3 sites x 5 days). For each member of the 5-member panel, a total of 60 data points (15 runs x 4 replicates) were generated using 1 lot of assay kit. The reproducibility panel comprised of a negative sample, 2 multi-analyte samples prepared at 3x Limit of Detection (LoD), and 2 multi-analyte samples prepared at 10x LoD. For the target composition of the 2 MA samples, refer to the table in *Matrix Equivalency*. As the performance of NxTAG RPP + SARS-CoV-2 assay with negative simulated matrix (NSM) was demonstrated to be equivalent to the performance with negative clinical matrix (NCM) in the matrix equivalency study, all sample preparation for this study were prepared using NSM as the sample matrix.

The results for site-to-site reproducibility for NxTAG RPP + SARS-CoV-2 are in *Table 35*. The results demonstrated reproducibility of the NxTAG RPP + SARS-CoV-2 assay across 3 sites with an overall percent agreement of 99.6% for all analytes at all test levels across all samples, sites, operators, and days.

Table 35. NxTAG® RPP + SARS-CoV-2 Site-to-Site Reproducibility Results

Target		Agreement with Expected Results				
	Concentration	Site 1	Site 2	Site 3	Overall (All Sites)	
Respiratory Syncytial Virus B	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	99.2% (119/120)
	10x LoD	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)	

Target	Concentration	Agreement with Expected Results				
		Site 1	Site 2	Site 3	Overall (All Sites)	
SARS-CoV-2	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	100% (120/120)
	10x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	
Human Bocavirus	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	99.2% (119/120)
	10x LoD	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)	
Mycoplasma pneumoniae	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	99.2% (119/120)
	10x LoD	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)	
Influenza A	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	100% (120/120)
	10x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	
Influenza A 2009 H1N1	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	100% (120/120)
	10x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	
Parainfluenza 1	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	100% (120/120)
	10x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	
Coronavirus OC43	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	100% (120/120)
	10x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	

Target		Agreement with Expected Results				
	Concentration	Site 1	Site 2	Site 3	Overall (All Sites)	
Adenovirus C	3x LoD	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)	99.2% (119/120)
	10x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	
Negative	N/A	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	100% (60/60)
Overall Agreement with Expected Results (all analytes and concentrations)						99.6% (1136/1140)

Operator-to-Operator Repeatability

Operator-to-operator repeatability testing was performed to assess the total variability of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay across operators and testing days. Two (2) operators at 1 site tested a 5-member reproducibility panel in 4 replicates on 5 non-consecutive days, for a total of 10 runs (2 operators x 1 sites x 5 days). For each member of the 5-member panel, a total of 40 data points (10 runs x 4 replicates) were generated using one (1) lot of assay kit. The operator-to-operator repeatability testing used the same sample panel as the site-to-site reproducibility testing. For the target composition of the 2 MA samples, refer to the table in *Matrix Equivalency*. The results for operator-to-operator repeatability for NxTAG RPP + SARS-CoV-2 are in *Table 36*. The results demonstrated repeatability of the NxTAG RPP + SARS-CoV-2 assay between two operators with an overall percent agreement of 100% for all analytes at all test levels across all samples and days.

Table 36. NxTAG® RPP + SARS-CoV-2 Within Lab (Operator-to-Operator) Repeatability Results

Target	Concentration	Agreement with Expected Results			
		Site 1	Site 2	Overall (All Sites)	
Respiratory Syncytial Virus B	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
SARS-CoV-2	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Human Bocavirus	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Mycoplasma pneumoniae	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	

Target	Concentration	Agreement with Expected Results			
		Site 1	Site 2	Overall (All Sites)	
Influenza A	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Influenza A 2009 H1N1	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Parainfluenza 1	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Coronavirus OC43	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Adenovirus C	3x LoD	100% (20/20)	100% (20/20)	100% (40/40)	100% (80/80)
	10x LoD	100% (20/20)	100% (20/20)	100% (40/40)	
Negative	N/A	100% (20/20)	100% (20/20)	100% (40/40)	100% (40/40)
Overall Agreement with Expected Results (all analytes and concentrations)					100% (760/760)

Lot-to-Lot Reproducibility

Lot-to-lot reproducibility testing was performed to assess the total variability of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay across 3 independent lots of assay kits. One (1) operator tested a 3- member reproducibility panel in 20 replicates on 3 different assay kit lots. For each member of the 3-member panel, a total of 60 data points (3 assay kit lots x 20 replicates) were generated. The lot-to-lot reproducibility panel was a subset of the site-to-site reproducibility test panel, consisting of a negative sample and 2 multi-analyte samples prepared at 3x LoD. The results for lot-to-lot reproducibility for NxTAG RPP + SARS-CoV-2 are in *Table 37*. The results demonstrated reproducibility of the NxTAG RPP + SARS-CoV-2 assay across three independent lots of assay kit with an overall percent agreement of 100% for all analytes across all samples.

Table 37. NxTAG® RPP + SARS-CoV-2 Lot-to-Lot Reproducibility Results

Target	Concentration	Agreement with Expected Results			
		Site 1	Site 2	Overall (All Sites)	
Respiratory Syncytial Virus B	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)

Target	Concentration	Agreement with Expected Results			
		Site 1	Site 2	Overall (All Sites)	
SARS-CoV-2	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Human Bocavirus	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Mycoplasma pneumoniae	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Influenza A	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Influenza A 2009 H1N1	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Parainfluenza 1	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Coronavirus OC43	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Adenovirus C	3x LoD	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Negative	N/A	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
Overall Agreement with Expected Results (all analytes)					100% (600/600)

Within-Run Repeatability

The within-run repeatability was assessed for total variability of the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) assay within 1 assay run. The within-run repeatability was assessed using the data generated on assay kit Lot 1 during lot-to-lot reproducibility study. The results for within-run repeatability for NxTAG RPP + SARS-CoV-2 are in *Table 38*. The results demonstrated repeatability of the NxTAG RPP + SARS-CoV-2 assay between 20 replicates of sample within one assay run with an overall percent agreement of 100% for all analytes across all samples.

Table 38. NxTAG® RPP + SARS-CoV-2 Within-Run Repeatability Results

Target	Concentration	Agreement with Expected Results
Respiratory Syncytial Virus B	3x LoD	100% (20/20)
SARS-CoV-2	3x LoD	100% (20/20)
Human Bocavirus	3x LoD	100% (20/20)
Mycoplasma pneumoniae	3x LoD	100% (20/20)
Influenza A	3x LoD	100% (20/20)
Influenza A 2009 H1N1	3x LoD	100% (20/20)

Target	Concentration	Agreement with Expected Results
Parainfluenza 1	3x LoD	100% (20/20)
Coronavirus OC43	3x LoD	100% (20/20)
Adenovirus C	3x LoD	100% (20/20)
Negative	3x LoD	100% (20/20)
Overall Agreement with Expected Results (all analytes)		100% (200/200)

Sample Carryover/Cross-Contamination

A Carryover/Cross-Contamination study was performed to evaluate the likelihood of carryover and cross-contamination for the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG RPP + SARS-CoV-2) Assay. SARS-CoV- 2 and two representative pathogen targets (viral: Parainfluenza 1 and bacterial: *Mycoplasma pneumoniae*) were prepared at high concentrations and extracted adjacent to negative samples (negative simulated matrix, NSM) in an alternating pattern. The extracted nucleic acid samples were tested on NxTAG RPP + SARS-CoV-2 assay in a checkerboard arrangement. No carryover and cross-contamination was observed as the NxTAG RPP + SARS-CoV-2 assay generated 100% expected results (for example: 100% target positivity for the respective high positive target samples and 0% target positivity for the negative samples) (*Table 39*).

Table 39. Summary of Carryover/Cross-Contamination Study Results for NxTAG® RPP + SARS-CoV-2

Sample Name	Organism	Testing Concentration	Target Positivity	Agreement with Expected Results
CoV-HP	SARS-CoV-2	1.00E+06 Copies/mL	100% (24/24)	100.00%
CoV-N	Negative	N/A	0% (0/24)	100.00%
PIV1-HP	Parainfluenza 1	1.00E+05 TCID50/mL	100% (24/24)	100.00%
PIV1-N	Negative	N/A	0% (0/24)	100.00%
Mpneumo-HP	*Mycoplasma pneumoniae*	1.00E+06 CCU/mL	100% (24/24)	100.00%
Mpneumo-N	Negative	N/A	0% (0/24)	100.00%

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