



**EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Healgen Rapid Check COVID-19/Flu A&B Antigen Test
DECISION SUMMARY**

I. Background Information:

A De Novo Number

DEN240029

B Applicant

Healgen

C Proprietary and Established Names

Healgen Rapid Check COVID-19/Flu A&B Antigen Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SCA	Class II	21 CFR 866.3987 – Multi-analyte respiratory virus antigen detection test	Microbiology

II. Submission/Device Overview:

A Purpose of Submission

De Novo request for evaluation of automatic class II designation for the Healgen Rapid Check COVID-19/Flu A&B Antigen Test

B Measurand

Influenza type A and type B nucleoprotein and SARS-CoV-2 nucleocapsid antigens

C Type of Test

Qualitative Lateral flow Immunoassay

III. Indications for Use:

A Intended Use(s):

See Indications for Use below

B Indication(s) for Use:

The Healgen Rapid Check COVID-19/Flu A&B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older.

All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2 or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should therefore seek follow-up care from their healthcare provider.

Positive results do not rule out co-infection with other respiratory pathogens and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up.

C Special conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

Not Applicable

IV. Device/System Characteristics:**A Device Description:**

The Healgen Rapid Check COVID-19/Flu A&B Antigen Test is an immunochromatographic assay that uses highly sensitive monoclonal antibodies to detect nucleoprotein antigens from SARS-CoV-2, influenza virus types A and B in anterior nasal swab (ANS) samples from symptomatic individuals. The test device is composed of a plastic housing, known as a cassette that contains a test strip with the following parts: sample pad, reagent pad, reaction membrane, and absorbing pad. The reagent pad contains colloidal gold conjugated with monoclonal antibodies (mAb) specific to SARS-CoV-2, Influenza A, and Influenza B target proteins. The reaction membrane contains different analyte specific antibodies to capture the target protein-gold-mAb complexes at the respective test lines. Excess liquid and reagents are absorbed by the absorbing pad. The Healgen Rapid Check COVID-19/Flu A&B Antigen Test does not use biotin-Streptavidin/avidin chemistry in any of the steps for coupling reagents.

B Principle of Operation:

When the test sample is added into the sample well (S) of the cassette, mAb conjugates dried in the reagent pad are dissolved and migrate along with the sample, across the reaction lines on the membrane. The reaction lines contain different antibodies that are also analyte specific and bind

to the target protein-gold-mAb complexes and immobilize them on the membrane, resulting in a visible red test line.

Results completely develop after 15 minutes. Reactions for each virus occur independently at their respective locations on the test reaction membrane. If the sample contains influenza type A or B antigens, a pink-to-red test line (A or B) will develop; if SARS-CoV-2 antigens are present, a pink-to-red test line (T) will develop. The procedural control lines (C) must always appear. Healgen Rapid Check COVID-19/Flu A&B Antigen Test is validated for testing direct samples without transport media.

The technical principle for influenza A virus and influenza B virus is identical to that of the SARS-CoV-2 antigen test strip as shown in figure 1 below.

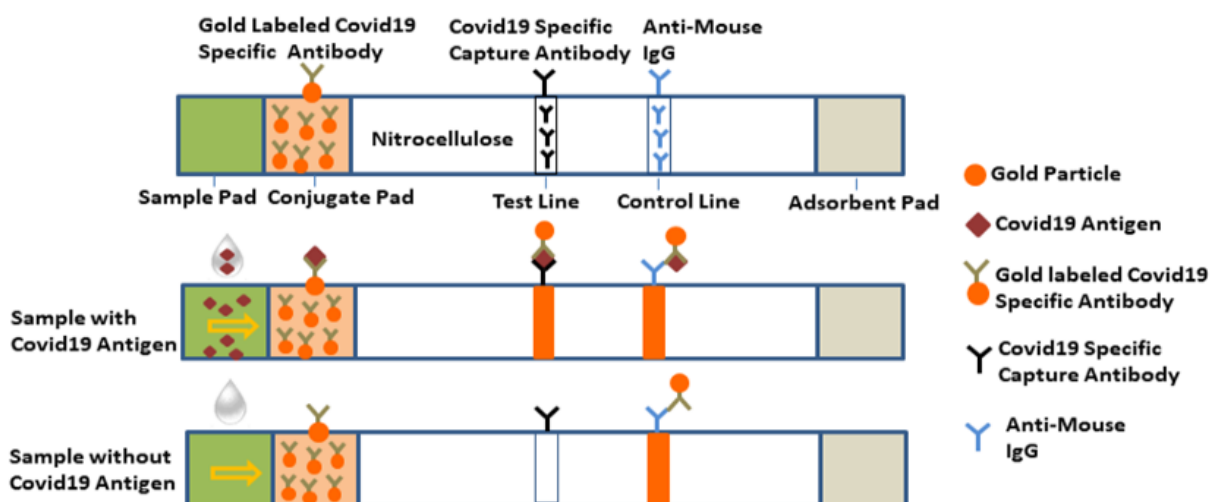
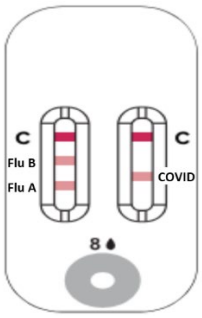
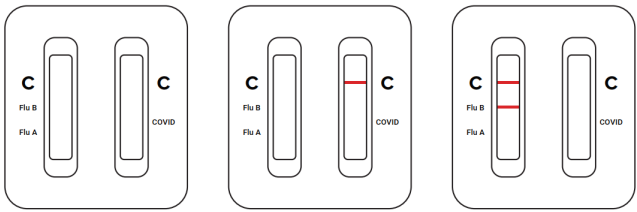
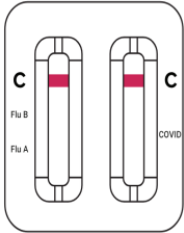


Figure 1: Schematic of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test Strip

Both the test strips enclosed in the test device independently feature an internal control, denoted directly on the test device as “C” for user interface. Test strip specific control lines are needed to indicate that each respective test strip is working adequately in each lay user performed test. The control line contains goat anti-mouse IgG antibodies that capture the excess gold-labeled mouse antibody preloaded in the reagent pad. The controls must be positive for a sample to provide a valid result to demonstrate that the test reagents are functional and correctly performed. If the control line is not detected, the sample result is invalid. Test results are displayed as Positive, Negative, or Invalid.

C Interpretation of Results:

The qualitative results of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test are visually interpreted by the user. Examples of the positive, negative, and invalid results interpretations are provided within the “Interpreting the Result” section of the QRI. Individuals can scan a QR code within the QRI. This code directs the test user to a complete list of test result interpretations prepared and provided online at: <https://www.healgen.com/covid19-influenza-a-b>. Results interpretation is described in the below figure.

<p>Visual for Positions of Result Lines</p>	 <p> C = Control Line Flu A, Flu B, COVID = Test Line indicating presence/absence of Flu B = Influenza B Flu A = Influenza A COVID = SARS-CoV-2 </p>
<p>Invalid (No Result)</p> <p>A valid result must display a pinkish-red Control Line in the control region ‘C’ of both the result windows. If no or only one window of the test cassette shows a pinkish-red Control Line in the control region ‘C’, the assay is invalid and cannot be interpreted no matter the presence of any positive Test lines in the cassette windows.</p> <p>Invalid tests should be repeated with a new test.</p>	<p style="text-align: center;">Invalid</p> <p style="text-align: center;">Missing ‘C’ line on ONE or BOTH strips</p>  <p>Note: The 3 images displayed are examples only; additional invalid outcomes are possible and can include test strips with or without test lines for Flu A, Flu B and/or COVID.</p>
<p>Negative Result</p> <p>A negative sample will produce a single pinkish-red Control Line in the control region ‘C’ of the window, indicating a negative result. This Control Line means that the detection part of the test was done correctly, sample was added, but no COVID-19 antigen was detected.</p>	<p style="text-align: center;">Valid Negative Result</p> <p style="text-align: center;">BOTH “C” lines must be PRESENT</p> 
<p>Positive Result</p> <p>A positive specimen will produce a single pinkish-red Test Line and a single pinkish-red Control Line. This means that COVID-19 antigen was detected and that the detection part of the test performed correctly. Specimens with low levels of antigen may give a faint Test Line.</p>	<p style="text-align: center;">Valid Positive Result</p> <p style="text-align: center;">BOTH “C” lines must be PRESENT</p>

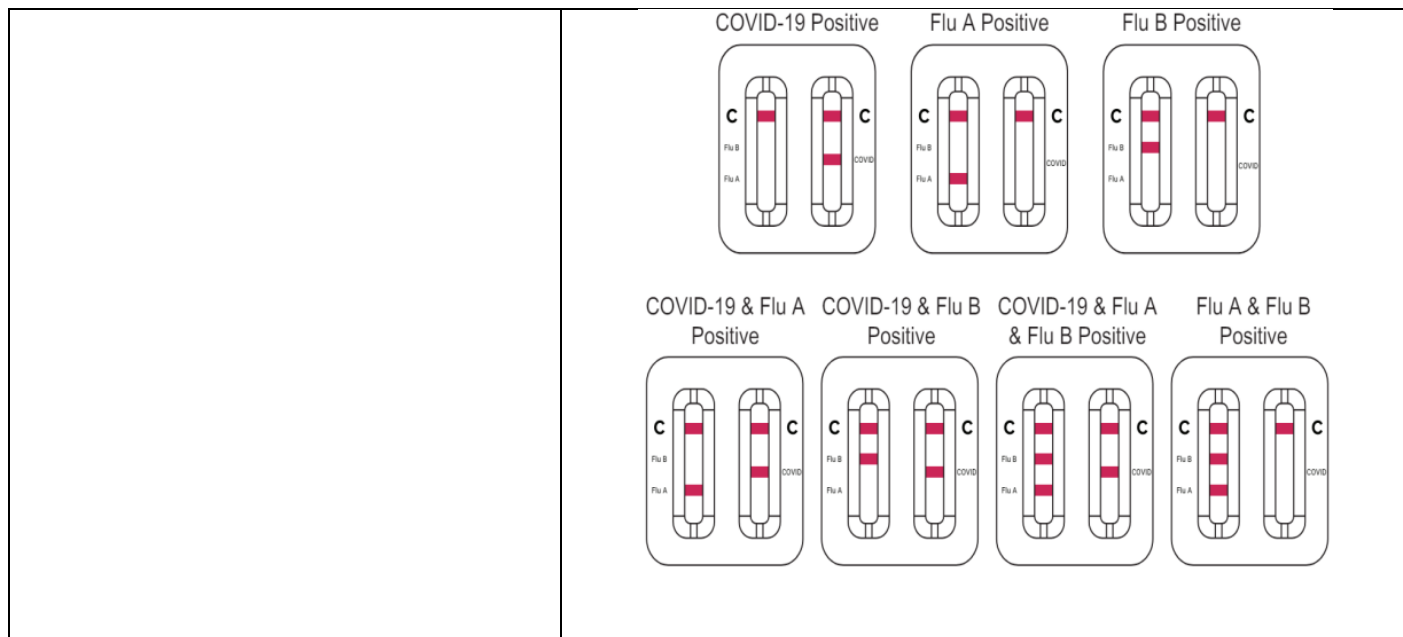


Figure 2: Results Interpretation

V. Standards/Guidance Documents Referenced:

Document Number	Title	Publishing Organization
EP25-A	Evaluation of Stability of In Vitro Diagnostics Reagents	CLSI
EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures	CLSI
EP12-A2	User Protocol for Evaluation of Qualitative Test Performance	CLSI
ISO 15223-1	Medical Devices – Symbols to be used with information to be supplied by the manufacturer	ANSI AAMI ISO
ISO 10993-5:2009	Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity	ANSI AAMI ISO
ISO 10993-10:2010	Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization	ANSI AAMI ISO
ISO 11135:2014	Sterilization of health-care products – Ethylene oxide – Requirements for the development, validation and routine control of a sterilization process for medical devices	ANSI AAMI ISO
ISO 10993-7	Biological evaluation of medical devices —Part 7: Ethylene oxide sterilization residuals.	ANSI AAMI ISO

VI. Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

The Precision study for the Healgen Rapid Check COVID-19/Flu A&B Antigen Test was evaluated in two different in-house studies using the same 3 lots of test kits and the same operators. The strains used for testing were PROtrol inactivated SARS-CoV-2 lineage BA.5; omicron variant, PROtrol inactivated influenza A/Guangdong-Maonan/SWL1536/19, and PROtrol inactivated influenza B/Washington/02/19.

Study 1 was conducted by 2 trained operators. Three sample levels (2X LoD co-spiked, 5X LoD co-spiked and Negative Pooled Nasal Wash) were tested on each day, one replicate per run, per operator, and per lot of devices. Two (2) runs (morning and afternoon) were conducted each day per operator, per lot, per day. This exact testing scheme was carried out over 10 days (same 3 sample levels tested, on the same 3 lots, by the same 2 operators, in 2 runs per day). This resulted in 120 total tests per sample level. All samples were randomized and blinded for each day. For all three lots and operators, the results for this study shown in the Table 1 below were identical and concordant with the expected results.

Study 2 was specifically conducted to further evaluate potential differences between lots. The study used negative samples (without virus analytes) and very low positive samples at 0.75x LoD, commonly referred to as high negative sample. Samples were prepared near the C95 concentration for all three analytes and were randomized and blinded. This supplemental precision testing was carried out over 3 days only, but otherwise followed the same study design as above. This resulted in 72 total tests per analyte and sample level (24 replicates for each analyte with each lot). Data from this testing are integrated into Table 1 below.

The random errors of the testing procedure across different days and runs, paired with an operator's ability to read the line intensity for samples with very low analyte concentration (commonly referred to as 'high negative samples') is expected to confound lot-specific variability and to have a significant impact on the precision estimates for high negative samples such as the 0.75 x LoD sample tested in this second part of the precision assessment. This is supported by the stratified data for study 2 that demonstrated imprecision also between runs and days (data not shown).

Taken together, the results of both precision assessments demonstrate a test precision and a lot-to-lot precision that are consistent with the expectations for the analyte concentration in the samples, the test's technology, and the test's LoD. The between-lot variability does not impact low concentrated samples equal to or above 2 x LoD of the test.

Table 1: Summary Results for Lot-Lot Precision study (Operators 1 and 2 Combined)

Sample	Analyte	Lot 1		Lot 2		Lot 3		Total Percent Lot-to-Lot Agreement	95% CI
		Count*	% Agreement	Count*	% Agreement	Count*	% Agreement		
Negative	SARS-CoV-2	0/64	100%	0/64	100%	0/64	100%	100%	98.0-100%
	Flu A	0/64	100%	0/64	100%	0/64	100%	100%	98.0-100%
	Flu B	0/64	100%	0/64	100%	0/64	100%	100%	98.0-100%
0.75 x LoD	SARS-CoV-2	20/24	83.3%	22/24	91.7%	17/24	70.8%	81.9%	71.5-89.1%
	Flu A	15/24	62.5%	15/24	62.5%	15/24	62.5%	62.5%	50.9-72.8%
	Flu B	18/24	75.0%	17/24	70.8%	14/24	58.3%	68.0%	56.6-76.7%
2 x LoD	SARS-CoV-2	40/40	100%	40/40	100%	40/40	100%	100%	93.9-100%
	Flu A	40/40	100%	40/40	100%	40/40	100%	100%	93.9-100%
	Flu B	40/40	100%	40/40	100%	40/40	100%	100%	93.9-100%
5 x LoD	SARS-CoV-2	40/40	100%	40/40	100%	40/40	100%	100%	93.9-100%
	Flu A	40/40	100%	40/40	100%	40/40	100%	100%	93.9-100%
	Flu B	40/40	100%	40/40	100%	40/40	100%	100%	93.9-100%

* The total number of replicates included in this table is different for the different sample concentrations due to the study being performed in two parts. Please refer to the study description above for more information.

2. Linearity:

This is a qualitative test without numerical data output and linearity is not applicable.

3. Analytical Specificity/Interference:

Cross-reactivity and Microbial Interference:

Cross Reactivity and Microbial Interference studies were conducted to determine if other respiratory pathogens/flora that could be present in a direct nasal swab samples could cause a false-positive test result or interfere with a true positive result. A panel of viruses, bacteria, fungi, and pooled nasal wash was used for these studies. Final target organism concentrations were 1.0×10^5 PFU/mL/ 1×10^5 TCID₅₀/mL for viruses, and 1.0×10^6 cfu/mL for bacteria and fungi. When the target concentration was not achievable due to the titer of the stock culture, the highest concentration possible was tested without dilution. Dilutions for cross-reactivity testing were made in pooled negative swab matrix in saline (NCM Saline). Each organism was tested in replicates of three (3) without SARS-CoV-2/ FluA/FluB present in the sample. All testing was randomized and blinded.

Organisms that did not cause a false-positive result were further evaluated for microbial interference by testing PNW spiked with low-level UV inactivated SARS-CoV-2, live Flu A virus, and live Flu B virus isolate (3X single analyte LoD) in the presence of potentially interfering organism at a high titer in triplicate. If interference was observed at the level tested, an additional titration study was performed to determine the highest microorganism concentration that does not produce interference with the Healgen Rapid Check COVID-19/Flu A&B Antigen test device.

Neither cross-reactivity nor interference was observed for any of the organisms at the concentrations tested with the Healgen Rapid Check COVID-19/Flu A&B Antigen test device.

The summary of cross-reactivity and microbial interference results are shown in the table below.

Table 2: Summary of Cross-reactivity and Microbial Interference Results

Organism	Concentrations Tested	Units	Cross-Reactivity	Microbial Interference
SARS-CoV-1	1.25E+05	PFU/ml	ND*	ND
MERS-coronavirus	1.47E+05	TCID ₅₀ /mL	ND	ND
Human coronavirus OC43	7.00E+05	TCID ₅₀ /mL	ND	ND
Human coronavirus 229E	1.58E+05	TCID ₅₀ /mL	ND	ND
Human coronavirus NL63	8.00E+04	TCID ₅₀ /mL	ND	ND
Human coronavirus HKU1 ^a	1:20 dilution	NA	NA	ND
Adenovirus, Type 1 (Adenoid 71)	2.23E+05	TCID ₅₀ /mL	ND	ND
Adenovirus Type 7, Type 7A (Species B)	1.58E+05	TCID ₅₀ /mL	ND	ND
Cytomegalovirus, Strain AD-169	7.05E+04	TCID ₅₀ /mL	ND	ND
Epstein Barr Virus, Strain B95-8	1.83E+06	CP/mL	ND	ND
Human Metapneumovirus (hMPV), Strain TN/91-316	3.50E+05	TCID ₅₀ /mL	ND	ND
Parainfluenza virus 1, Strain FRA/29221106/2009	2.00E+05	TCID ₅₀ /mL	ND	ND
Parainfluenza virus 2, Strain Greer	1.75E+05	TCID ₅₀ /mL	ND	ND
Parainfluenza virus 3, Strain C243	7.00E+05	TCID ₅₀ /mL	ND	ND
Parainfluenza virus 4, Strain N/A	2.39E+05	TCID ₅₀ /mL	ND	ND
Enterovirus Type (e.g. 68), Species D Type 68	2.23E+05	TCID ₅₀ /mL	ND	ND
Respiratory syncytial virus A, Strain A-2	3.50E+05	TCID ₅₀ /mL	ND	ND
Respiratory syncytial virus B, Strain CH93(18)-18	2.29E+05	TCID ₅₀ /mL	ND	ND
Rhinovirus 1A, Strain N/A	7.05E+04	TCID ₅₀ /mL	ND	ND
Bordetella pertussis, Strain A639	2.50E+08	CFU/mL	ND	ND
Candida albicans, Strain Z006	6.03E+06	CFU/mL	ND	ND
Chlamydia pneumoniae, Strain Z500	4.33E+06	IFU/mL	ND	ND
Corynebacterium xerosis	2.30E+07	CFU/mL	ND	ND
Escherichia coli, Strain mcr-1	1.79E+08	CFU/mL	ND	ND
Hemophilus influenzae, type b; Eagan	9.68E+06	CFU/mL	ND	ND
Lactobacillus sp., Lactobacillus Acidophilus, Strain Z048	1.21E+07	CFU/mL	ND	ND
Legionella spp pneumophila, Strain Philadelphia-1	6.50E+06	CFU/mL	ND	ND
Moraxella catarrhalis, Strain 59632	2.50E+08	CFU/mL	ND	ND
Mycoplasma pneumoniae, Strain PI 1428	2.50E+07	CFU/mL	ND	ND
Mycobacterium tuberculosis avirulent, Strain H37Ra-1	4.15E+06	CFU/mL	ND	ND
Neisseria meningitidis, serogroup A	3.43E+06	CFU/mL	ND	ND
Neisseria sp. Elongata Z071	2.68E+08	CFU/mL	ND	ND
Pneumocystis jirovecii,	1.30E+07	CFU/mL	ND	ND

Organism	Concentrations Tested	Units	Cross-Reactivity	Microbial Interference
Strain W303-Pji				
Pseudomonas aeruginosa, Strain N/A	3.45E+08	CFU/mL	ND	ND
Staphylococcus aureus Protein A producer, e.g., Cowan strain, NCTC 8530 [S11]; Cowan's serotype 1	2.60E+08	CFU/mL	ND	ND
Staphylococcus epidermidis (PCI 1200)	9.00E+07	CFU/mL	ND	ND
Streptococcus salivarius, Strain C699 [S30D]	1.01E+06	CFU/mL	ND	ND
Streptococcus pneumoniae, Strain Z022	1.81E+07	CFU/mL	ND	ND
Streptococcus pyogenes, Strain MGAS 8232	7.50E+07	CFU/mL	ND	ND
Measles, Strain Edmonston	8.48E+05	TCID ₅₀ /mL	ND	ND
Mumps (Isolate 1)	8.48E+05	TCID ₅₀ /mL	ND	ND

*ND – Not Detected

^a1:10 dilution of cultured stock HKU1 sample from Emory

Competitive Interference:

Competitive interference of the test's analytes was tested with different combinations of low (3x LoD) and high concentrations of Flu A, Flu B and SARS-CoV-2 spiked together onto a swab and then tested with one lot of Healgen Rapid Check COVID-19/Flu A&B Antigen test device. The study used inactivated SARS-CoV-2 but live influenza A and B virus strains.

The table below summarizes the results of the competitive interference study. For each condition tested all three replicates tested at the low target analyte condition tested positive in the presence of a second target analyte at high concentrations. No false positive results were observed for analytes not present in the sample.

Table 3: Competitive Interference Results Summary

	Analyte Concentration Added to Sample* (No. positive replicates / No. of total replicates)		
	Flu A	Flu B	SARS-CoV-2
Analyte Concentration Added Results	High 3/3	Low 3/3	- 0/3
Analyte Concentration Added Results	High 3/3	- 0/3	Low 3/3
Analyte Concentration Added Results	Low 3/3	High 3/3	- 0/3
Analyte Concentration Added Results	- 0/3	High 3/3	Low 3/3
Analyte Concentration Added Results	Low 3/3	- 0/3	High 3/3
Analyte Concentration Added Results	- 0/3	Low 3/3	High 3/3

* SARS-CoV-2 strain – 1X LoD - 3.95E+02 TCID₅₀/mL

Flu A – H3N2:A/Darwin/6/2021 – 1X LoD – 2.09E+02 TCID₅₀/mL

Flu B – Yamagata: B/Florida/4/2006 – 1X LoD-1.46E+01 TCID₅₀/mL

Exogenous and Endogenous Interference Study

The Healgen Rapid Check COVID-19/Flu A&B Antigen test was evaluated for performance in the presence and absence of potentially interfering substances that might be present in a respiratory specimen. Interfering substances testing was performed using a panel of endogenous and exogenous substances tested at concentrations listed in the below table.

Negative specimens were evaluated in triplicates to confirm that the potentially interfering substances would not cause false positive results with the test.

Negative clinical matrix (pooled nasal wash) was co-spiked with SARS-CoV-2 USA WA1/2020, Flu A H1N1pdm09/A Victoria/4897/2022, and Flu B Yamagata/B/Florida/4/2006, and then mixed 1:1 with interfering substance. Final concentration for each analyte was 3x LoD (based on the established single analyte LoD). Negative nasal wash has been demonstrated to be equivalent to the anterior nasal swab matrix in a matrix equivalency study. Testing was performed in triplicate to confirm that SARS-CoV-2, Flu A and Flu B could still be detected if the test substances were present in the sample. All testing was randomized and blinded. Test results are summarized in the table below.

With the exception of Flu Mist Quadrivalent live influenza vaccine, none of the substances caused a false-positive test result in unspiked samples. While the presence of Flu Mist Quadrivalent live influenza vaccine at 15% v/v concentration did not interfere with the detection of true positive results of the 3x LoD co-spiked samples, the vaccine also resulted in positive results for Flu A and Flu B (as expected based on the composition of the vaccine). Hand sanitizer cream lotion and hand sanitizer 80% ethanol fast drying at 15% v/v showed false negative results for Flu B, but detected all analytes at 7.5% v/v.

Table 4: Interfering Substances Study Results

Interfering Substance	Concentration	Cross-Reactivity (no analyte) (# pos/ # total)			Interference (3x co-spiked analyte LoD) (# pos/ # total)		
		SARS-CoV-2	Flu A	Flu B	SARS-CoV-2	Flu A	Flu B
Human Whole Blood (EDTA tube)	4% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Leukocytes	1.67 x 10 ⁶ cells/mL	0/3	0/3	0/3	3/3	3/3	3/3
Throat Lozenges (Menthol/Benzocaine)	3 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Mucin, bovine submaxillary gland	2.5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Zinc (Therazinc throat Spray)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Naso GEL (NeilMed)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Drops (Phenylephrine)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Spray (Oxymetazoline)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Spray (Cromolyn)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Corticosteroid (Dexamethasone)	1 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Corticosteroid (Fluticasone Propionate)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3

Interfering Substance	Concentration	Cross-Reactivity (no analyte) (# pos/ # total)			Interference (3x co-spiked analyte LoD) (# pos/ # total)		
		SARS-CoV-2	Flu A	Flu B	SARS-CoV-2	Flu A	Flu B
Nasal gel (Galphimia glauca, Histanium hydrochloricum, Luffa operculata, Sulfur)	1.25%	0/3	0/3	0/3	3/3	3/3	3/3
Homeopathic allergy relief (Histaminum hydrochloricum)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Zicam nasal spray (Galphimia glauca, Luffa operculata)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal spray (Alkalol)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Sore Throat Phenol Spray	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Tobramycin	4 µg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Mupirocin	10 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Anti-viral drug (Remdesvir)	10 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Tamiflu (Oseltamivir)	5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
FluMist (Quadrivalent/Live)	15% v/v	0/3	3/3	3/3	3/3	3/3	3/3
	0.15% v/v	0/3	0/3	0/3	NA	NA	NA
Zanamivir	282 ng/mL	0/3	0/3	0/3	3/3	3/3	3/3
Biotin	3500 ng/mL	0/3	0/3	0/3	3/3	3/3	3/3
Body & Hand Lotion	0.5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Body Lotion, with 1.2% dimethicone	0.5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Lotion	5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Sanitizer with Aloe, 62% ethyl alcohol	5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Sanitizer cream lotion	15% v/v	0/3	0/3	0/3	3/3	3/3	0/3
	7.5% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Sanitizer, 80% ethanol	15% v/v	0/3	0/3	0/3	3/3	3/3	0/3
	7.5% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand soap liquid gel	10% w/v	0/3	0/3	0/3	3/3	3/3	3/3

4. Assay Reportable Range:

This section is not applicable as this device is a qualitative assay.

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

Internal Controls:

Both the test strips enclosed in the test device independently feature an internal control, denoted directly on the test device as "C" for user interface. Test strip specific control lines are needed to indicate that each respective test strip is working adequately in each lay user performed test. The control line contains IgG antibodies that capture the excess gold-labeled mouse antibody preloaded in the reagent pad. These controls have to be positive for all valid test results to

demonstrate that the test reagents are functional, and the tests correctly performed. If the control lines are not detected, the sample result is invalid.

External Controls:

External Control testing is not performed by lay users and is therefore not applicable to OTC tests. External controls are therefore not included in the test kit.

Stability

Real Time Stability:

Three lots of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test kits were subjected to a summer profile (40°C for 8 hours and then 30°C for 4 hours) and then a winter profile (-10°C for 8 hours and then 18°C for 4 hours) to simulate the anticipated shipping/handling times and temperatures expected for unopened kits, after which they were stored at 2-8°C or 30±3°C, respectively. The test panel comprised of negative clinical matrix, 2x LoD and 5x LoD of inactivated SARS-CoV-2, and live Flu A and Flu B viruses. Testing was performed at time 0 (baseline) and month 1, 3, 6, 9, 12, 15, and 18. Testing will continue for months 21, 24 and 27. All study data are 100% concordant with expected results and support a shelf-life of up to 15 months. The shelf life will be updated as additional passing time points will become available.

Open Kit Stability Study:

In this study, the amount of time a test device can be left outside of its packaging was assessed using a test panel comprised of five (5) negative samples (clinical matrix: PNW) and five (5) co-spiked low positive samples (2X single analyte LoD of SARS-CoV-2, Flu A, and Flu B co-spiked together into PNW). PNW was demonstrated to be equivalent to negative nasal swab matrix in a matrix equivalency study. Device packaging was opened and testing was performed at zero (0) hours to establish baseline. Thereafter, devices were stored for one-hour and two-hour, respectively at 30±1°C (the worst-case condition for a room temperature claim). All study data before and after storage of the open kits were 100% concordant with the expected results.

Transport Stability:

Simulated winter and summer transport temperature conditions were used to evaluate the worst-case shipping and handling of unopened components of the Healgen COVID-19/Flu A&B Ag Combo Rapid test over an extended period. The functional performance of Healgen's test device is assessed by comparing the pre- (T0) and post-distribution (Td) results of a test panel comprised of pooled negative nasal wash (PNW) samples and co-spiked low positive samples (3X single analyte LoD with SARS-CoV-2, Flu A, and Flu B, together contrived in PNW). PNW was demonstrated to be an equivalent negative clinical matrix to negative nasal swab matrix in a matrix equivalency study. All results were as expected for all time points.

6. Detection Limit:

Single Analyte LoD:

The LoD of the device was performed to determine the lowest detectable concentration of SARS-CoV-2, influenza A and influenza B at which at least 95% of all true positive replicates are consistently detected as positive. The LoD was assessed for each analyte in two parts, a preliminary range finding study, followed by a confirmatory LoD study. A preliminary LoD was

determined by first testing serial ten-fold dilutions of live influenza A and B, and inactivated SARS-CoV-2 virus stocks diluted into either pooled negative swab matrix (PNSM) or pooled nasal wash (PNW) in 3 replicates per dilution. Single analyte virus dilutions (50 µL/swab) were each spiked onto dry sterile swabs and tested per the IFU.

The preliminary LoD results for each individual virus strain is shown in below tables.

Table 5: Preliminary LoD - SARS-CoV-2

Isolate/Lineage	SARS-CoV-2 (TCID ₅₀ /mL)	SARS-CoV-2 (TCID ₅₀ /Swab)	#Positive/ # Total	# of device lots tested
USA-WA1-2020 (UV inactivated)	3.16E+05	1.58E+04	3/3	1
	3.16E+04	1.58E+03	3/3	
	3.16E+03	1.58E+02	3/3	
	1.58E+03	7.90E+01	3/3	
	7.90E+02	3.95E+01	3/3	
	3.95E+02	1.98E+01	3/3	
	3.16E+02	1.58E+01	2/3	
	1.58E+02	7.90E+00	0/3	
USA-WA1-2020 (Heat inactivated)	3.09E+06	1.5E+05	3/3	1
	3.09E+05	1.5E+04	3/3	
	3.09E+04	1.5E+03	3/3	
	3.09E+03	1.5E+02	3/3	
	1.5E+03	7.5E+01	1/3	
	3.09E+02	1.5E+01	0/3	
USA/COR-22-063113/2022 (BA.5, Omicron variant)	2.19E+03	1.09E+02	9/9	3
	1.09E+03	5.45E+01	9/9	
	5.47E+02	2.73E+01	4/9	
	2.19E+02	1.09E+01	2/3	

Table 6: Preliminary LoD - Influenza A

Isolate/Lineage	Strain	SARS-CoV-2 (TCID ₅₀ /mL)	SARS-CoV-2 (TCID ₅₀ /Swab)	#Positive/ # Total	# of device lots tested
H3N2	A/Darwin/6/2021	4.17E+04	2.09E+03	3/3	1
		4.17E+03	2.09E+02	3/3	
		4.17E+02	2.09E+01	3/3	
		2.09E+02	1.05E+01	3/3	
		1.04E+02	5.20E+00	3/3	
		5.21E+01	2.61E+00	3/3	
		4.17E+01	2.09E+00	2/3	
		4.17E+04	2.09E+03	0/3	
H1N1	pdm09:A/Victoria/48 97/2022	2.02E+04	1.01E+03	3/3	1
		2.02E+03	1.01E+02	3/3	
		2.02E+02	1.01E+01	3/3	
		1.01E+02	5.05E+00	1/3	
		5.05E+01	2.53E+00	0/3	
		2.53E+01	1.27E+00	0/3	
		2.02E+01	1.01E+00	0/3	

Isolate/Lineage	Strain	SARS-CoV-2 (TCID ₅₀ /mL)	SARS-CoV-2 (TCID ₅₀ /Swab)	#Positive/ # Total	# of device lots tested
	A/California/07/2009 pdm09	2.1E+05	5.85E+02	3/3	1
		2.1E+04	5.85E+01	3/3	
		2.1E+03	5.85E	3/3	
		1.05E+03	5.25E+01	3/3	
		5.25E+02	2.6E+01	1/3	
		2.1E+02	1.05E+01	0/3	
	Guangdong- Maonan/SWL 1536/19	5.62E+01	2.81	9/9	3
		5.62	2.81E-01	0/3	

Table 7: Preliminary LoD - Influenza B

Isolate/Lineage	Strain	SARS- CoV-2 (TCID ₅₀ / mL)	SARS-CoV-2 (TCID ₅₀ /Swa b)	#Positive/# Total	# of device lots tested
Yamagata	B/Florida/4/2006	1.17E+04	5.85E+02	3/3	1
		1.17E+03	5.85E+01	3/3	
		1.17E+02	5.85E+00	3/3	
		5.85E+01	2.93E+00	3/3	
		2.93E+01	1.47E+00	3/3	
		1.46E+01	7.30E-01	3/3	
		1.17E+01	5.85E-01	1/3	
Victoria	B/Washington/02/2019	3.16E+05	1.58E+04	3/3	1
		3.16E+04	1.58E+03	3/3	
		3.16E+03	1.58E+02	3/3	
		1.58E+03	7.90E+01	1/3	
		7.90E+02	3.95E+01	0/3	
		3.95E+02	1.98E+01	0/3	
		3.16E+02	1.58E+01	0/3	
	B/Washington/02/2019 (PROtrol inactivated)	1.75E+04	8.75E+02	9/9	3
		8.75E+03	4.37E+02	0/3	
		1.75E+03	8.75E+01	0/3	
	B/Florida/78/2015	1.7E+06	8.5E+04	3/3	1
		1.7E+05	8.5E+03	3/3	
		1.7E+04	8.5E+02	3/3	
		8.5E+03	4.25E+02	1/3	
		1.7E+03	8.5E+01	0/3	

LoD confirmatory testing was then performed individually for each virus by testing 20 replicates at the virus' preliminary (1X) LoD concentration, as determined above. For the LoD to be confirmed, at least 95% of the replicates ($\geq 19/20$) needed to test positive. Results of the LoD confirmation testing for each virus are summarized in the table below.

Table 8: Confirmatory LoD

Analyte	Isolate/ Lineage	Strain	LoD Concentration (TCID ₅₀ /mL)	LoD Concentration (TCID ₅₀ /swab)	#Positive /# Total	# device lots tested
SARS-CoV-2	USA-WA1/2020 (UV inactivated)	NA	3.95E+02	1.98E+01	20/20	1
	USA-WA1/2020 (Heat inactivated)	NA	3.09E+03	1.5E+02	60/60	3
	USA/COR-22- 063113/2022 (BA.5, Omicron variant)	NA	1.09E+03	5.45E+01	58/60	3
Flu A	H3N2	Darwin/6/21	2.09E+02	1.05E+01	20/20	1
	H1N1	Victoria/4897/22	2.02E+02	1.01E+01	20/20	1
		A/California/07/200 9 pdm09	1.05E+03	5.25	60/60	3
		Guangdong- Maonan/SWL 1536/19 (PROtol inactivated)	5.62E+01	2.81	60/60	3
Flu B	Yamagata	Florida/04/06	1.46E+01	7.30E-01	20/20	1
	Victoria	Washington/02/19	1.58E+03	7.90E+01	20/20	1
	Victoria	Washington/02/19 (PROtol inactivated)	1.75E+04	8.75E+02	58/60	3
	Victoria	B/Florida/78/2015	1.7E+04	8.5E+02	60/60	3

Co-spiked LoD:

After the single analyte LoDs were established for the candidate device, co-spiked LoD equivalency testing with all three test analytes present in the sample, was conducted to characterize performance with samples that contain more than one analyte at low concentrations. All analytes that are successfully detected by the candidate device when co-spiked at their single analyte LoD, may be co-spiked into positive sample/s used in the analytical studies.

Based on the individual analyte specific 1x LoDs, co-spiked samples were prepared by mixing all three viruses (one strain each of SARS-CoV-2, Flu A and Flu B). The 1x co-spiked LoD concentration was tested with the candidate device in twenty (20) replicates and was considered confirmed (i.e., equivalent to the established single analyte LoD) if $\geq 19/20$ replicates were positive for concentrations within 2x LoD of the established single analyte LoD.

The Healgen Rapid check COVID-19/Flu A&B Antigen Test demonstrated co-spike equivalency for all analytes, SARS-CoV-2, Flu A and Flu B, to their respective established single analyte 1X LoD. The summary of the co-spike LoD is shown in the below table.

Table 9: Summary of Co-Spike Equivalency LoD Results

Virus	Fold LoD	LoD Concentration (TCID ₅₀ /mL)	LoD Concentration per Swab (TCID ₅₀ /swab)	# Positive Replicates
SARS-CoV-2 (USA-WA1/2020)	1X	3.95 x 10 ²	2.0 x 10 ¹	20/20
Flu A H1N1 (pdm09:A/Victoria/4897/2022)	1X	2.02 x 10 ²	1.0 x 10 ¹	20/20
Flu B Yamagata (B/Florida/4/2006)	1X	1.46 x 10 ¹	7.3	20/20

NIBSC 21/368 -WHO International Standard:

The sponsor tested the sensitivity of the test with the 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) spiked into pooled negative swab matrix (PNSM). The unitage of this material has an assigned value of 5,000 International Units of SARS-CoV-2 antigen per ampoule when reconstituted per instructions. A 2-fold dilution series was made to determine the preliminary LoD, which was measured using one device lot and triplicate measurements (n=3). The measurements were done by adding 50µl of each dilution directly to the test swab and processing the sample per the test's QRI. The preliminary LoD was determined to be 250 IU/ml (or 12.5 IU/swab).

The LoD confirmatory study was performed using 20 replicates (n=20) per dilution. The lowest concentration at which a minimum of 95% of results were positive was confirmed to be 250 IU/ml or 12.5 IU/Swab as shown below.

Table 10: LOD with the 1st WHO International Standard for SARS-CoV-2 Antigen (NIBSC code:21/368)

Dilution (IU/ml)	Preliminary LoD		Confirmatory LoD		
	Dilution (IU/swab)	Results	Dilution (IU/ml)	Dilution (IU/swab)	Results
4x10 ³	200	3/3			
2x10 ³	100	3/3			
1x10 ³	50	3/3			
5x10 ²	25	3/3	5x10 ²	25	20/20
2.5x10 ²	12.5	3/3	2.5x10 ²	12.5	19/20
1.25x10 ²	6.25	2/3	1.25x10 ²	6.25	10/20
6.25x10 ¹	3.125	0/3			

High-dose Hook Effect Study:

The hook effect study was conducted to evaluate if high levels of antigen present in the sample could result in a false negative test result. In this study, 50µL of the highest concentration possible for UV inactivated SARS-CoV-2 virus stock and for live influenza A and influenza B virus stocks were spiked onto sterile swabs for triplicate measurements, and swabs were tested on the device per IFU of the candidate device.

Testing showed no hook effect for SARS-CoV-2, Flu A, Flu B at the concentrations listed in the table below.

Table 11: Summary of High Dose Hook Effect Results

Virus	Strain	Subtype / Lineage	Virus Concentration [TCID ₅₀ /mL*]	Virus Concentration [TCID ₅₀ /swab]	# Positive/ # Tested
SARS-CoV-2	USA-WA1/2020	N/A	3.16E+06	1.58E+05	3/3
Influenza A	A/Victoria/4897/2022	H1N1	2.02E+05	1.01E+04	3/3
Influenza A	A/Darwin/6/2021	H3N2	4.17E+05	2.09E+04	3/3
Influenza B	B/Washington/02/2019	Victoria	3.16E+06	1.58E+05	3/3
Influenza B	B/Florida/4/2006	Yamagata	1.17E+05	5.85E+03	3/3

*Concentration in the sample solution applied to dry swab

Inclusivity Study:

Inclusivity testing was conducted to determine if the candidate device can detect different strains of SARS-CoV-2, Flu A and Flu B.

The Healgen Rapid Check COVID-19/Flu A&B Antigen Test employs the identical test strip as included in the previously cleared COVID-19-only Healgen Rapid COVID-19 Antigen Test (K232377). Inclusivity data for SARS-CoV-2 as obtained for K232377 therefore apply equally to the Healgen Rapid Check COVID-19/Flu A&B Antigen Test. Data derived from testing of commercially obtained Alpha (B.1.1.7), delta (B.1.617.2), omicron (B.1.1.529), beta (B.1.351), gamma (P1) and kappa (B.1.617.1) SARS-CoV-2 virus strains demonstrated the test strip's inclusivity of all tested variants at low concentrations (see FDA's Decision Summary for [K232377 \(fda.gov\)](https://www.fda.gov/medical-devices/covid-19-tests/healgen-rapid-check-covid-19-flu-a-b-antigen-test)).

A selection of temporal, geographic and genetically diverse Influenza A and B strains were tested on the Healgen Rapid Check COVID-19/Flu A&B Antigen Test for inclusivity. A series of ten-fold dilutions of each virus strain was made in pooled negative nasal matrix (PNSM). For each replicate tested in the study, 50µL of the dilution was pipetted on a fresh sterile swab. Once the ten-fold breakpoint was established for each of the strains, an additional series of three two-fold dilutions was made from the lowest positive ten-fold dilution of each virus and triplicates were tested to demonstrate inclusivity. Contemporary strains (within the past 5 years) were prioritized over older strains.

The lowest concentrations that tested positive for relevant influenza virus strains by the candidate device are shown in the table below.

Table 12: Inclusivity Results – Minimal Detectable Concentrations of Flu Variants

Virus	Virus Strains	Concentration	Units
Flu A - H1N1	A/ California/04/2009	2.80E+03	TCID ₅₀ /mL
	A/ Brisbane/02/2018	1.51E+02	TCID ₅₀ /mL
	A/ Michigan/45/2015	9.30E+00	TCID ₅₀ /mL
	A/ Guangdong-Maonan/SWL 1536/2019	1.04E+03	TCID ₅₀ /mL
	A/ NY/03/2009	2.29E+04	TCID ₅₀ /mL
	A/ Indiana/02/2020	9.70E+06	CEID ₅₀ /mL
	A/Wisconsin/588/2019	1.4E+04	FFU/mL
	A/ Sydney/5/2021	4.80E+03	TCID ₅₀ /mL
	A/ Hawaii/66/2019	3.70E+07	CEID ₅₀ /mL
	A/ Wisconsin/67/2022	1.05E+03	TCID ₅₀ /mL
	A/New York/21/2020	2.6E+05	FFU/mL

Virus	Virus Strains	Concentration	Units
Flu A – H3N2	A/Tasmania/503/2020	6.5E+04	FFU/mL
	A/Hong Kong/2671/2019	3.1E+06	CEID ₅₀ /mL
	A/Hong Kong/45/2019	1.5E+04	FFU/mL
	A Alaska/01/2021	1.50E+04	FFU/mL
	A/Indiana/08/2011	8.10E+02	TCID ₅₀ /mL
Flu A– H1N1	A/Ohio/09/2015	7.0E+05	CEID ₅₀ /mL
Flu A– H1N2	A/Minnesota/19/2011	8.00E+06	CEID ₅₀ /mL
Flu A– H5N1	A/mallard /Wisconsin/2576/2009	2.10E+05	GE/mL
	A/mallard /Wisconsin/2576/2009	800,000	CEID ₅₀ /mL
	A/Bovine/Ohio/B24OSU-439/2024	1,550	TCID ₅₀ /mL
	A/duck/Guangxi/S11002/2024	3.38E+05	EID ₅₀ /mL
Flu A– H5N6	A/duck/Guangxi/S10888/2024	7.90E+05	EID ₅₀ /mL
Flu A– H5N8	A/goose/Liaoning/S1266/2021	1.69E+05	EID ₅₀ /mL
Flu A– H7N3	A/northern pintail/Illinois/10OS3959/2010	7.0E+05	CEID ₅₀ /mL
Flu B –Victoria Lineage	B/ Brisbane/60/2008	6.45E-01	TCID ₅₀ /mL
	B/Colorado/6/2017	5.85E+00	TCID ₅₀ /mL
	B/Texas/02/2013	6.13E+00	TCID ₅₀ /mL
	B/ Michigan/01/2021	2.85E+03	TCID ₅₀ /mL
Flu B – Yamagata Lineage	B/Texas/06/2011	8.00E+05	CEID ₅₀ /mL
	B/Utah/09/2014	1.26E+02	TCID ₅₀ /mL
	B/Wisconsin/1/10	1.78E+01	TCID ₅₀ /mL
Flu B – non-Victoria, non-Yamagata	B/Maryland/1/1959	1.69E+03	CEID ₅₀ /mL

Assay Cut-Off:

Not applicable as this is a qualitative visually read assay without numeric raw data.

B Comparison Studies:

Method Comparison:

Please refer to section VI.C (Clinical Studies) below for the clinical validation, regarding the method comparison studies.

Matrix Comparison:

The candidate device is only intended for qualitative detection of nucleocapsid protein antigen from SARS-CoV-2, and nucleoprotein from Flu A and Flu B in direct anterior nasal swab specimens. As no other sample types are claimed herein, a matrix comparison study is not applicable.

However, the sponsor performed the matrix equivalency study between pooled negative nasal swab matrix (PNSM) and the surrogate pooled negative nasal wash (PNW) that was used in multiple analytical studies. The data demonstrated equivalent performance of the test with both matrices.

C Clinical Study:

1. Clinical Performance Assessment:

A multi-center, prospective clinical study was conducted with lay users to assess the performance of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test in detecting nucleoprotein antigens extracted from COVID-19, influenza virus types A and B in self-collected and self-tested anterior nasal swab samples. The study only enrolled subjects with two or more symptoms of respiratory infection consistent with COVID-19 or influenza. Ten clinical sites across the U.S. conducted the study from February to April 2024. Both the comparator and the candidate test used anterior nasal swab samples and the collection order was alternated by study subject. Comparator test samples were collected by health care professionals at the clinical study sites and inserted into Universal Transport Media per the IFU of the comparator test. Samples were then sent to a central site for testing with highly sensitive RT-PCR tests separately detecting SARS-CoV-2 and Flu A/B. Samples for the candidate antigen test were collected per the test's quick reference instructions and were either self-collected by a lay user aged ≥ 14 years or collected by an adult (parent/guardian) from individuals aged 2 to <14 years.

There were 1,156 symptomatic subjects enrolled with a symptom onset between 0 and 5 days. Of those, 1,122 subjects were evaluable for Flu A/B, and 1,097 subjects were evaluable for SARS-CoV-2. The study cohort included 16% low positive samples. The age of participants ranged from 2 years old to 89 years old, with a mean of 36 years. The education level of subjects ranged from high school diploma to doctorate degree. The demographics of the subjects involved in the clinical study are shown in the table below.

Table 13: Subjects Demographics

	Subjects (by lay- user collection and testing (N=178))	Self-collecting and testing (N=944)	Overall (N=1122)
Age Group			
≥ 2 - <14 years of age	171 (96.1%)	0 (0.0%)	171 (15.2%)
≥ 14 - <24 years of age	6 (3.4%)	147 (13.1%)	153 (13.6%)
≥ 24 - <65 years of age	0 (0.0%)	710 (75.2%)	710 (61.6%)
≥ 65 years of age	1 (0.6%)	87 (9.2%)	88 (7.8%)
Age: Mean (SD)	8.2 (6.0)	41.3 (15.9)	36 (19.1)
Age: Median [Min, Max]	8 [2, 71]	40 [14, 89]	35 [2, 89]
Sex at Birth			
Female	83 (46.6%)	550 (58.3%)	633 (56.4%)
Male	95 (53.4%)	394 (41.7%)	489 (43.6%)
Ethnicity			
Hispanic/Latino	108 (60.7%)	427 (45.2%)	535 (47.7%)
Not Hispanic/Latino	70 (39.3%)	517 (54.8%)	587 (52.3%)
Race			

	Subjects (by lay- user collection and testing (N=178))	Self-collecting and testing (N=944)	Overall (N=1122)
American Indian or Alaskan Native	1 (0.6%)	2 (0.2%)	3 (0.3%)
Asian	0 (0.0%)	4 (0.4%)	4 (0.4%)
Black or African American	8 (4.5%)	145 (15.4%)	153 (13.6%)
Native Hawaiian/Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)
White	161 (90.4%)	730 (77.3%)	891 (79.4%)
Unknown/Prefer not to answer	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other (Mixed race/biracial)	8 (4.5%)	63 (6.7%)	71 (6.3%)
Education Level (testers and subjects self-collecting and testing)			
Less than high school diploma	34 (19.1%)	116 (12.3%)	150 (13.3%)
High school diploma	89 (50.0%)	392 (41.5%)	481 (42.9%)
Some college, but no degree	25 (14.0%)	160 (16.9%)	185 (16.5%)
Associate Degree (e.g., AA, AS)	19 (10.7%)	95 (10.1%)	114 (10.1%)
Bachelor's Degree (e.g., BA, BBA and BS)	10 (5.6%)	135 (14.3%)	145 (12.9%)
Master's Degree (e.g., MA, MS and Meng)	0 (0.0%)	27 (2.9%)	27 (2.9%)
Professional Degree (e.g., MD, DDS, JD)	0 (0.0%)	7 (0.7%)	7 (0.7%)
Doctorate (e.g., PhD, EdD)	1 (0.6%)	2 (0.2%)	3 (0.3%)
Other	0 (0.0%)	10 (1.1%)	10 (1.1%)
Total	178 (100.0%)	944 (100.0%)	1,122 (100.0%)

Results obtained with the Healgen Rapid Check COVID-19/Flu A&B Antigen Test were compared to the results obtained with highly sensitive RT-PCR comparator tests giving rise to the following performance estimates:

Table 14: Clinical Performance for Detection of SARS-CoV-2

SARS-CoV-2	Comparator Positives	Comparator Negatives	Total
Candidate Positives	69	10	79
Candidate Negatives	6	1,012	1,018
Total	75	1,022	1,097
Positive Percent Agreement (PPA): 92.0% (69/75) - 95% CI: 83.6% - 96.3%			
Negative Percent Agreement (NPA): 99.0% (1012/1012) - 95% CI: 98.2% - 99.5%			

Results for SARS-CoV-2 were also analyzed stratified by the days post symptom onset (DPSO) and are presented in Table 15 below.

Table 15: Clinical Performance for Detection of SARS-CoV-2 stratified by DPSO

DPSO*	Subject Number tested	Investigational Device Positive	Comparator Device Positive	% Positive (by Comparator)	PPA (95% CI)
Day 0	24	0	0	0.0%	NA
Day 1	180	12	13	7.2%	92.3% (66.7% - 99.6%)
Day 2	341	15	17	5.0%	88.2% (65.7% - 96.7%)
Day 3	285	16	17	6.0%	94.1% (73.0% - 99.7%)
Day 4	194	21	21	10.8%	100.0% (84.5% - 100.0%)
Day 5	73	5	7	9.6%	71.4% (35.9% - 91.8%)
Total	1097	69**	75	6.8%	92.0% (83.6% - 96.3%)

* DPSO: Days Post Symptom Onset

**False positive results on the investigational device were excluded from the analysis

Table 16: Clinical Performance for Detection of Influenza A

FLU A	Comparator Positives	Comparator Negatives	Total
Candidate Positives	49	1	50
Candidate Negatives	4	1068	1072
Total	53	1069	1122
Positive Percent Agreement (PPA): 92.5% (49/53) - 95% CI: 82.1% - 97.0%			
Negative Percent Agreement (NPA): 99.9% (1068/1069) - 95% CI: 99.5% - 100.0%			

Table 17: Clinical Performance for Detection of Influenza B

FLU B	Comparator Positives	Comparator Negatives	Total
Candidate Positives	38	1	39
Candidate Negatives	4	1079	1083
Total	42	1080	1122
Positive Percent Agreement (PPA): 90.5% (38/42) - 95% CI: 77.9% - 96.2%			
Negative Percent Agreement (NPA): 99.9% (1079/1080) - 95% CI: 99.5% - 100.0%			

Clinical Sensitivity:

Please refer to Section VI.C (Clinical Studies) above for the clinical validation. The PPA for the test for each analyte is as follows:

SARS-CoV-2: 92.0% (69/75) - 95% CI: 83.6% - 96.3%

Flu A: 92.5% (49/53) - 95% CI: 82.1% - 97.0%

Flu B: 90.5% (38/42) - 95% CI: 77.9% - 96.2%

Clinical Specificity:

Please refer to Section VI.C (Clinical Studies) above for the clinical validation. The NPA for the test for each analyte is as follows:

SARS-CoV-2: 99.0% (95% CI: 98.2% - 99.5%)
Flu A: 99.9% (95% CI: 99.5% - 100.0%)
Flu B: 99.9% (95% CI: 99.5% - 100.0%)

Serial Testing:

Due to the high clinical sensitivity of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test (i.e., above 90%), serial (repeat) antigen testing, previously recommended by FDA for SARS-CoV-2 Antigen tests, is not needed for this test.

2. Usability Assessment:

Usability study was conducted to evaluate the usability of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test and to evaluate the labeling and comprehension of the subject test QRI when performed by lay users in a simulated home environment. The study was conducted as part of the clinical study from February – April 2024. The first fifteen (15) or more subjects from the clinical study who were self-collecting and testing, and the first fifteen (15) or more subjects collecting a sample and performing the testing on another subject (child or adult), were selected to participate in the human factors assessment. The demographics of the usability study is tabulated below.

Table 18: Demographics of the Usability Study Population

Factor	Lay-user (Tester) collection and testing (N=25)	Self-collecting and testing (N=26)	Overall (N=51)
Subject Age			
Mean (SD)	7.4 (2.9)	46.5 (15.9)	27.3 (22.8)
Median [Min, Max]	8 [2, 13]	50 [15, 77]	15 [2, 77]
Subject Age Group			
≥2-13 years of age	25 (100.0%)	0 (0.0%)	25 (49.0%)
≥14-17 years of age	0 (0.0%)	2 (7.7%)	2 (3.9%)
18-29 years of age	0 (0.0%)	2 (7.7%)	2 (3.9%)
30-50 years of age	0 (0.0%)	9 (34.6%)	9 (17.6%)
51-65 years of age	0 (0.0%)	11 (42.3%)	11 (21.6%)
>65 years of age	0 (0.0%)	2 (7.7%)	2 (3.9%)

The human factors assessment portion of the study was completed per the protocol. Fifty-one (51) subjects (26 self-collecting and testing, and 25 lay users collecting and testing from another) were enrolled in the human factors assessment. Evaluation of the human user experience indicated high usability of the investigational test. All subjects who participated found the instructions to be clear and easy to follow and found the sample collection easy to perform, as well as having no difficulty reading the test results. Additionally, 97.5% (796 out of 816 tests) of the mock tests were interpreted correctly. Overall, 96.8% of all critical tasks associated with sample collection and the running of the Healgen COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) were performed correctly. Additionally, 87.6% of all non-critical tasks were

performed correctly. The human factors assessment met the predetermined targets for the percentage of critical and noncritical tasks performed correctly as shown in the table below.

Table 19: Usability Study Results

Steps	Tasks performed correctly	Total number of tasks	Percentage of tasks performed correctly
Critical	642	663	96.8%
Non- Critical	134	153	87.6%
Total	776	816	95.1%

3. Lay User Readability Assessment:

All 51 subjects who participated in the human factors assessment (Usability study) also interpreted a panel of mock investigational tests with various results that reflected the test concentration levels at 1.9x and 5x the limits of detection (LOD) in a blinded and random fashion. Each panel of mock tests included 16 investigational tests with various negative and positive results for each analyte. The percentage of total human factor subjects with vision impairment is 39.2% (20/51). Vision impairments encountered in study subjects are listed in the table below with their respective frequency of occurrence. The study did not include individuals with any of the following: macular degeneration, color blindness, diabetic retinopathy, glaucoma, cataracts, or amblyopia/strabismus. The overall accuracy of the results interpreted by the lay users is 97.5% (796/816): 95% CI (96.2% - 98.4%).

Table 20: Vision Impairment of Readability Study Subjects

Vision Impairment	# of Subjects	Percentage of total human factors subjects with vision impairment (N=51)
Near sightedness only (with lens prescription)	6	11.8%
Far sightedness only (with lens prescription)	6	11.8%
Astigmatism	1	2.0%
More than one visual impairment condition	7	13.7%
Total subjects/testers with vision impairment	20	39.2%

The comparison of result interpretation data between lay users with and without visual impairment is tabulated below.

Table 21: Lay User Readability Study Results

Mock Results Type	Accuracy of Mock Test Interpretations [%]	
	Subjects without vision impairment (N=31)	Subjects with vision impairment (N=20)
1.9x LoD - Flu A+ & Flu B+	100.0%	100.0%
1.9x LoD - COV-19+ /Flu A+	93.5%	100.0%

Mock Results Type	Accuracy of Mock Test Interpretations [%]	
	Subjects without vision impairment (N=31)	Subjects with vision impairment (N=20)
1.9x LoD - COV-19+ /Flu A+ & Flu B+	93.5%	100.0%
1.9x LoD - COV-19+ /Flu B+	90.3%	100.0%
1.9x LoD - COV+	93.5%	100.0%
1.9x LoD - Flu A+	100.0%	100.0%
1.9x LoD - Flu B+	96.8%	100.0%
5x LoD - Flu A+ & Flu B+	100.0%	100.0%
5x LoD - COV-19+ /Flu A+	96.8%	100.0%
5x LoD - COV-19+ /Flu A+ & Flu B+	100.0%	100.0%
5x LoD - COV-19+ /Flu B+	96.8%	95.0%
5x LoD - COV+	100.0%	100.0%
5x LoD - Flu A+	100.0%	95.0%
5x LoD - Flu B+	100.0%	100.0%
Invalid	93.5%	95.0%
Negative	93.5%	95.0%
Total	96.8%	98.8%

D Clinical Cut-Off:

Not Applicable. The candidate device is a qualitative assay with a visually read binary result without numeric raw data.

E Expected Values/Reference Range:

A patient sample is expected to be negative for SARS-CoV-2, influenza A and influenza B. This section is therefore not applicable.

F Other Supportive Performance Characteristics Data:

1. Variant Monitoring Plan:

To determine whether the Healgen Rapid Check COVID-19/Flu A/B Antigen Test can detect newly emerging variants, and/or to assess whether new mutations are impacting analytical sensitivity of the test performance, the sponsor provided the variant monitoring plan as described below:

- a. Monitoring SARS-CoV-2, Influenza A and B Sequence Data in GISAID database, WHO, NIH and other public health entities:
The updated sequence data for SARS-CoV-2, influenza a and influenza B variants from GISAID database, WHO, NIH and other public health entities will be downloaded and analyzed bimonthly for variant mutations in the target proteins with an allele frequency of $\geq 5\%$

- b. In silico analysis of antigenicity of the N proteins:
In silico monitoring of antigen variations caused by changes in aminoacid residues will be performed by analyzing linear epitopes through sequence alignments and structure prediction methods.
- c. Wet lab test on recombinant N proteins:
Full length recombinant mutated N proteins will be used for wet lab testing for VOC monitoring, including VOC/VOI/VUM SARS-CoV-2 strains as well as other strains with higher frequency mutation.
- d. Evaluation with SARS-CoV-2, Flu A and B variant viruses with External Quality Assessment schemes or commercially available viruses:
External quality assessment schemes or commercial resources will be used to determine possible performance effect of our test affected by mutations in the testing proteins. Additional validation tests using clinical samples will be conducted involving a comparative analysis of the results obtained through the candidate device, an FDA-approved PCR test and next-generation sequencing techniques to ensure the utmost accuracy and reliability of our testing device's performance assessment.

2. Frequently Asked Questions:

To improve user label comprehension, the labeling includes a Frequently Asked Questions (FAQ) section. The FAQ section was created to provide users information to adequately understand the meaning of the test results and test types as well as the accuracy of the test. The concepts covered in the FAQ section include:

- Meaning of the test results.
- When to re-test (e.g., following an invalid result). Difference between antigen and molecular test
- Accuracy of the test
- Follow-up for appropriate health management.

3. Hazard Analysis:

A comprehensive hazard analysis of the Healgen Rapid Check COVID-19/Flu A/B Antigen Test included identification of the potential hazard, likelihood of occurrence, severity of potential harm, hazard control measure(s), hazard control verification, and assignment of pre- and post-control risk levels. The elements considered included operator errors (i.e., human factors), sample and device handling and storage, and environmental factors.

Potential sources of errors that could adversely affect system performance were identified and mitigated through cautions in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies that evaluated the functionality of fail-safe mechanisms and stressed the functional limits of the test system (see below).

4. Fail-Safe Features:

The device features an internal control to minimize false results due to user error. The internal control monitors for grossly insufficient sample volume, adequate membrane wicking, sample flow, and the integrity of the detection reagents.

5. Flex Studies:

To assess the robustness and risk for false results of the test when deviating from the IFU/QRI test steps, flex studies were conducted that assessed all major aspects of the test procedure (sample volume, reading time, swab extraction time and procedure [delay in mixing and addition of the sample], sample hold time before and during processing) and variability of environmental test conditions that the test may be subjected to when in use (lighting, disturbance during use, temperature and humidity stress conditions). Testing was performed with negative PNW samples and low positive samples co-spiked with SARS-CoV-2, Flu A and Flu B virus into negative PNW at 2xLoD.

The results demonstrated that the test system is robust and that false results can be expected to be reasonably mitigated through labeling.

VII. **Proposed Labeling:**

The labeling supports the decision to grant the De Novo request for this device.

VIII. **Identified Risks and Mitigations:**

Risks to Health	Mitigation Measures
False Results	Certain labeling information including limitations, device descriptions, explanations of procedures, and performance information. Certain design verification and validation including documentation of device descriptions, certain analytical and clinical studies, and risk analysis. Certain strain monitoring and reporting strategies.
Failure to correctly interpret test results	Certain labeling information including limitations, device descriptions, explanations of procedures, and performance information. Certain design verification and validation including documentation of device descriptions, certain analytical and clinical studies, and risk analysis.
Failure to correctly operate the device	Certain labeling information including limitations, device descriptions, explanations of procedures, and performance information.

IX. **Benefit/Risk Assessment:**

A **Summary of the Assessment of Benefit:**

The primary probable benefit of the Healgen Rapid Check COVID-19/Flu A&B Antigen Test device is to facilitate easy-to-use rapid detection of respiratory viruses by the lay user in the

home environment using an adequately validated, well-performing, multi-analyte respiratory test, with the potential to test an entire household within the first 5 days of onset of symptoms. Home-based testing for the detection and differentiation of these viruses is addressing an unmet public health need because it offers several important advantages, foremost shortening the time to diagnosis and therefore treatment and isolation, as necessary. This reduces the risk of severe illness as well as virus transmission. Indirectly, this may lessen demand on overburdened public health and clinical laboratories during times of increased transmission. For persons facing particular barriers to accessing care outside the home, whether due to physical, cognitive, or demographic factors, at-home over-the-counter (OTC) tests may potentially help improve health equity in early diagnosis and treatment, and thereby improve outcomes of COVID-19, influenza A (Flu A), and influenza B (Flu B).

B Summary of the Assessment of Risk:

The risks associated with the device, when used as intended, are related to the risk of false results as a consequence of either performance issues of the test, and/or a failure of the operator to correctly operate the test or interpret the test results correctly.

False positive SARS-CoV-2, influenza A virus, and influenza B virus test results may lead to initiation of improper patient management (such as administration of unnecessary or wrong antiviral drugs with unintended complications) or failure to recognize and appropriately treat other respiratory illnesses that may resemble COVID-19 or influenza but are not caused by these viruses.

False negative SARS-CoV-2, influenza A virus, and influenza B virus test results may lead to unnecessary additional diagnostic evaluation or treatment, and delays in correct diagnosis possibly leading to missed opportunities to initiate time-sensitive treatment, and/or infection control measures to avoid transmission to additional persons. This may consequently lead to an increase in patient morbidity and mortality.

Visually interpreted tests also have a risk to be falsely interpreted as invalid when the actual result is a valid positive or valid negative result. In instances where a valid test result is falsely interpreted as invalid, this may lead to unnecessary delays while additional testing is sought, possibly leading to missed opportunities to initiate time-sensitive treatment, and/or infection control measures to avoid transmission to additional persons.

C Summary of the Assessment of Benefit-Risk:

The most serious risk of the device is a false negative result, leading to a missed or delayed diagnosis of SARS-CoV-2, influenza A virus or influenza B virus infection, or a false positive result, leading to improper patient management or failure to recognize and appropriately treat other respiratory illnesses resembling COVID-19 and influenza. However, the risk of false results is low, as evident from the results of the required device validation and verification, including analytical and clinical studies. In addition, this risk can be mitigated by the clear guidance provided in the labeling limitations, the clear procedural instructions in the labeling written at a 7th grade reading level, and the repeated and clear guidance in the labeling for the user to contact their healthcare provider if there is evidence of worsening disease or risk factors for severe disease are present.

The risk of failure to correctly interpret the test results of a visually read device is mitigated by clear pictorial and descriptive instructions in the result interpretation section of the labeling with important warning statements being emphasized in bolded text.

Equally, the risk of failure to correctly operate the device is also mitigated by certain labeling information, including the presence and emphasis (bolded text) of warnings and limitations within the procedural instructions, device descriptions, and performance sections.

Clinical and usability data suggest that errors will be uncommon and that the test can be accurately performed and results accurately interpreted. While general controls are not sufficient to mitigate the risks of this device, in light of the special controls, the benefits outweigh the risks for this device.

X. Conclusion:

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): SCA

Device Type: Multi-analyte respiratory virus antigen detection test

Class: II

Regulation: 866.3987