Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab)

INSTRUCTIONS FOR USE.

REF GCFC-525Sa

IVD

For Prescription Use Only.
For Emergency Use Authorization (EUA) Only.

INTENDED USE

The Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) is a lateral flow immunochromatographic assay intended for in vitro rapid, simultaneous qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly from anterior nasal swab specimens of individuals with signs and symptoms of respiratory infection consistent with COVID-19 within the first five (5) days of symptom onset when tested at least twice over three days with at least 48 hours between tests. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet the requirements to perform moderate, high, or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the simultaneous *in vitro* detection and differentiation of SARS-CoV-2, influenza A virus, and influenza B virus protein antigens, but do not differentiate, between SARS-CoV and SARS-CoV-2 viruses and are not intended to detect influenza C antigens.

These viral antigens are generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definitive cause of the disease.

All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out influenza or SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control measures such as isolating from others and wearing masks. Negative results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with each respiratory infection.

The Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) is only for *in vitro* diagnostic use under the Food and Drug Administration's Emergency Use Authorization. This product has not been cleared or approved.

SUMMARY

COVID-19 and influenza are acute and highly contagious viral infections of the respiratory tract. The causative agents of the diseases are immunologically diverse, single-strand RNA viruses known as SARS-CoV-2 viruses and influenza viruses, respectively. There are three types of influenza viruses: A, B and C. Type A viruses are the most prevalent and are associated with more serious disease whereas Type B infection is generally milder. Type C virus has never been associated with a large epidemic of human disease.

A patient can be infected with a single virus or co-infected with SARS-CoV-2 and one or more types of influenza viruses. These viral infections occur more often during the respiratory illness season (in the US this includes the fall and winter seasons) and the symptoms generally appear 3 to 7 days after the infection. Transmission for all of these viruses occurs easily through coughing and sneezing of aerosolized droplets from infected people, who may be either symptomatic or asymptomatic. For symptomatic patients, the main symptoms include fever, fatigue, dry cough, and loss of taste and smell. Nasal congestion, runny nose, sore throat, myalgia, and diarrhea were also associated symptoms.

Rapid diagnosis of SARS-CoV-2 and influenza A & B viral infection will help healthcare professionals treat patients and control these diseases more effectively.

PRINCIPLE

The Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) is an immunochromatographic assay that uses highly sensitive monoclonal antibodies to detect nucleocapsid protein antigens extracted from COVID-19, influenza virus types A and B with anterior nares swab samples.

The test device is a plastic housing, known as a cassette, containing two test strips, each composed of the following parts: sample pad, reagent pad, reaction membrane, and absorbing pad. The reagent pads contain colloidal gold conjugated with monoclonal antibodies (mAb) specific for SARS-COV-2, Influenza A, and Influenza B target proteins. When the test sample is added into the sample well (S) of the cassette, mAb conjugates dried in the reagent pad are dissolved and interact with the viruses' proteins in the sample (if present). These complexes migrate along the test strip and across the reaction lines on the membrane. The reaction line contains a second antibody specific to available target protein-mAb complexes with each of the virus antigens of the test, resulting in visible test lines for the viruses in the sample.

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 line (C) must always appear on both strips for the test to be valid. The Healgen® Rapid COVID-19 + Influenza A/B Antigen test is validated for testing direct samples without

transport media. The Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) does not use biotin-streptavidin/avidin chemistry in any of the steps for coupling reagents.

WARNINGS, PRECAUTIONS, AND SAFETY INFORMATION

1. For *in vitro* diagnostic use.
2. Read the instructions fully and carefully before performing the procedure. Failure to follow the instructions may result in inaccurate results.
3. In the USA, this product has not been FDA cleared or approved; but has been authorized by FDA under an Emergency Use Authorization. This product has been authorized only for the detection of proteins from SARS-CoV-2, Influenza A, and Influenza B, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
4. **Serial testing should be performed in individuals with SARS-CoV-2 negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.**
5. **Consistent with serial testing recommendations for SARS-CoV-2, for multi-analyte tests, symptomatic individuals who test positive for influenza A or B on the initial test but test negative for SARS-CoV-2 should be tested again in 48 hours to evaluate for co-infection with SARS-CoV-2 infection.**
6. Do not use the test kit after its expiration date.
7. Do not use the test if the pouch is damaged or open.
8. Test components are single use. Do not reuse the test cassette, processing solution, or swab.
9. Wear a safety mask or other face covering when collecting a specimen from another person.
10. This product is only for the detection of proteins from influenza A, influenza B, and SARS-CoV-2, not for any other viruses or pathogens.
11. For use with anterior nasal swab specimens only and not for use with viral transport media (VTM).
12. Do not open the test contents until ready for use. If the test cassette is open for an hour or longer, invalid test results may occur.
13. Inadequate or inappropriate sample collection, storage, or transport may yield false test results.
14. Testing should be performed in an area with good lighting.
15. **Do not read test results before 15 minutes or after 20 minutes. Results read before 15 minutes or after 20 minutes may result in false positive, false negative or invalid results.**
16. Dispose of all used materials in accordance with federal, state, and local regulatory requirements.
17. This test does NOT determine if you had COVID-19 in the past or if you have immunity.
18. **Keep the testing kit and kit components away from children and pets before and after use. Avoid contact with your skin, eyes, nose, or mouth. Do not ingest any kit components. The reagent solution contains harmful chemicals (see table below). If the solution contacts your skin, eyes, nose, or mouth, flush with large amounts of water. If irritation persists, seek medical advice: https://www.poisonhelp.org or 1-800-222-1222.**

Hazard Category (mixture)	Hazard Class	GHS Hazard Statement for mixture	Hazardous Ingredients (%)*
2	Skin irritation	Causes skin irritation (H315)	Tris (2.4%) 1, 2-Benzisothiazolin-3-One (0.04%)
2	Eye Irritation	Causes eye irritation (H320)	1, 2-Benzisothiazolin-3-One (0.04%) Tris (2.4%) Ethylenediamine ethoxylated propoxylated polymer (S9) (0.75%)

19. For more information on EUAs please visit: "https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization"
20. For the most up to date information on COVID-19, please visit: "http://www.cdc.gov/COVID19

STORAGE AND STABILITY

- Store the test kit between 36-86°F (2-30°C) in a place out of direct sunlight.
- Reagents and devices must be used at room temperature (59-86°F/15-30°C).
- The unsealed cassette is valid for 1hour. It is recommended to use the test kit immediately after opening. The expiration date is on the package.

MATERIALS PROVIDED

- 25 Sealed Test Cassettes
- 25 Sterile Nasal Swabs
- 25 Pre-filled Extraction Tubes
- 25 Extraction Tube Tips
- 2 Tube Holders
- 1 Instructions for Use
- 1 Healthcare Provider Fact Sheet
- 1 Patient Fact Sheet
- 1 Quick Reference Guide (QRG)

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or clock
- External Controls - Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) (Catalog no.: GCFC-PN2, GCFC-PN20 -Sold separately)

SPECIMEN COLLECTION AND PREPARATION

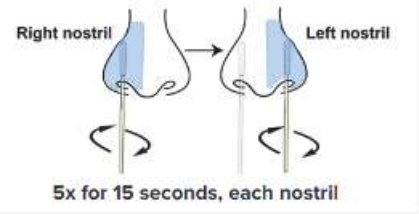
NOTE: Do not open the test contents until ready for use. If the test cassette is open for an hour or longer, invalid test results may occur. **When collecting a sample, only use the swab provided in the kit.** Allow the

test cassette, nasal swab and extraction tube to come to room temperature [15-30℃(59-86°F)] prior to testing.

1. Check the test's expiration date printed on the outer test packaging.
2. Assemble the tube holder, insert the extraction tube into the tube holder. Ensure it is stable and upright.
3. Tear off the sealing film on the extraction tube gently to avoid spilling the liquid.
4. Remove the test cassette from the sealed pouch and lay it on a clean, flat surface.
5. Remove the swab from the pouch. Carefully insert the sterile swab no more than 3/4 inch (1.5 cm) into the nostril.

   Note: *Be careful not to touch the swab tip (soft end) with hand.*

6. Slowly rotate the swab **at least 5 times** against the nostril wall for **at least 15 seconds**. Remove the swab and repeat in the other nostril using the same swab.

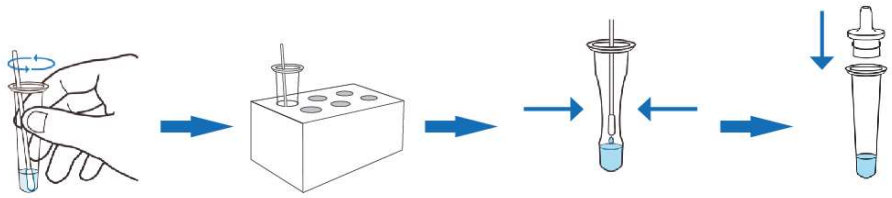


TEST PROCEDURE

1. Immerse the swab into the prefilled extraction tube and swirl the swab in the buffer. Ensure the sample is mixed thoroughly by **making at least 6 circles**.

   Note: *Sample must be mixed in the extraction buffer within 2 hours of sample collection.*

2. Leave the swab in the extraction tube for **1 minute**. A timer is recommended for this step.
3. After 1 minute, pinch the tip of the swab from the outside of the tube to remove any excess sample in the swab. Remove and discard the swab.
4. Hold the tube upright and insert the extraction tube tip into the tube opening. Ensure a tight fit to prevent leaking.

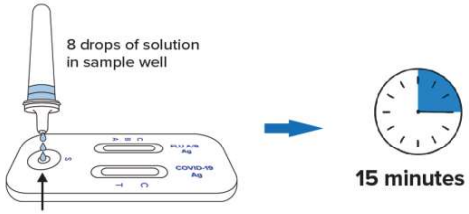


5. Invert the extraction tube and **squeeze 8 drops** of test sample into the sample well. Then discard the tube.

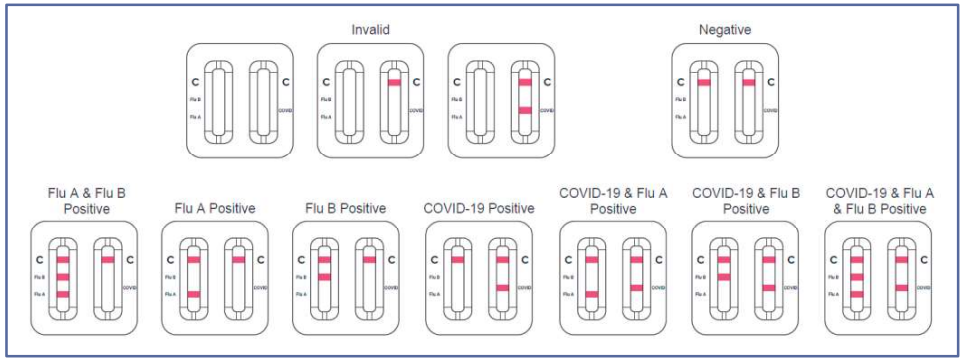
   Note: *Sample must be applied to the test cassette within one hour of completing step 1.*

6. Start timer. **Read results between 15 minutes and 20 minutes**.

   Note: *Interpreting results before 15 or after 20 minutes can give false results.*



INTERPRETATION OF RESULTS



Invalid Result: The test could not tell whether or not the patient has COVID-19, influenza A (Flu A), or influenza B (Flu B). The test needs to be repeated with a new kit and sample.

Note: The 3 images displayed are examples only; for additional invalid results, scan the QR code in the "Additional Information: Reading Results" section below.

Negative Result: The virus from COVID-19, Flu A, and/or Flu B were not detected in the sample. A negative result does not mean it is certain that the patient does not have COVID-19, Flu A and/or Flu B infection. There is a higher chance of false negative results with antigen tests compared to laboratory-based molecular tests. If the patient tested negative and continues to experience COVID-19, Flu A and/or Flu B-like symptoms, the patient should seek follow-up care with the healthcare provider.

Note: COVID-19 Negative (-) Result

To increase the chance that the negative result for COVID-19 is accurate, you should: Test again in 48 hours if the individual has symptoms on the first day of testing.

A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow up testing for SARS-CoV-2 with a molecular test or testing for other respiratory disease should be considered. If applicable, seek follow up care with the primary health care provider.

All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.

Positive Result: The COVID-19, Flu A, and/or Flu B virus(es) were detected in the patient sample. It is very likely that the patient has the respective infection(s) and are contagious. Please contact the healthcare provider or local health authorities and follow local guidelines for self-isolation. There is a small chance that this test can give a positive result that is incorrect (a false positive).

Note: COVID-19 Positive (+) Result

Repeat testing does not need to be performed if patients have a positive result at any time.

A positive test result means that the virus that causes COVID-19 was detected in the sample, and it is very likely the individual has COVID-19 and is contagious. Please contact the patient's doctor/primary care physician (if applicable) and the local health authority immediately and instruct your patient to adhere to the local guidelines regarding self- isolation. There is a very small chance that this test can give a positive result that is incorrect (a false positive).

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) should self-isolate and seek follow up care with their physician or healthcare provider as additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

SERIAL TESTING

Repeat Testing is needed to improve test accuracy for negative SARS-CoV-2 results. Please follow the table below when interpreting test results with symptoms. Serial (repeat) SARS-CoV-2 testing does not need to be performed if patients have a positive SARS-CoV-2 result.

Status on First Day of Testing	Day 0 (First Test)	Serial Testing ?	Day 2 (Second Test)	Final Interpretation
With Symptoms	SARS-CoV-2 (+) Influenza A and B (-)	NO	Not needed	Positive for COVID-19 Presumptive negative for Influenza
	SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (-)	Positive for COVID-19 Presumptive Negative for Influenza
	SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19 Positive for Influenza A and/or B
	SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (-) Influenza A and/or B (+)	Presumptive Negative for COVID-19 Positive for Influenza A and/or B
	SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (-) Influenza A and/or B (-)	Presumptive Negative for COVID-19 Presumptive Negative for Influenza
	SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19 Positive for Influenza A and/or B
	SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (-) Influenza A and/or B (-)	Presumptive Negative for COVID-19 Positive for Influenza A and/or B
	SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (-) Influenza A and/or B (+)	Presumptive Negative for COVID-19 Positive for Influenza A and/or B
	SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19 Positive for Influenza A and/or B
	SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (-)	Positive for COVID-19 Presumptive Negative for Influenza

Additional Information: Reading Results

Scan QR code for more information on reading results.

Webpage: https://www.healgen.com/covid19-influenza-a-b

LIMITATIONS

- The performance of this test is established based on the evaluation of a limited number of clinical specimens collected between June 2023 and June 2024. The clinical performance has not been established for all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of COVID-19 and influenza and their prevalence, which change over time.
- There is a higher chance of false negative results with antigen tests than with laboratory-based molecular tests due to the sensitivity of the test technology. This means that there is a higher chance this test will give a false negative result in an individual with COVID-19 as compared to a molecular test, especially in samples with low viral load.
- All COVID-19 and influenza A&B antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
- If the patient continues to have symptoms of COVID-19, and both the patient's first and second tests are negative, the patient may not have COVID-19 infection, however additional follow-up may be needed.
- If the test is positive, then proteins from the virus that causes COVID-19 or influenza infection have been found in the sample and the individual likely has a respiratory infection with SARS-CoV-2 or influenza.
- This test is read visually and has not been validated for use by those with impaired vision or color-impaired vision.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- Based on sequence analysis, a potential for cross-reactivity between the SARS-CoV-2 test and HKU1 exists. Wet testing for HKU1 coronavirus was not conducted and therefore, cross-reactivity between SARS-CoV-2 and HKU1 coronavirus cannot be ruled out.
- Use of Healgen COVID-19/Flu A&B Combo Rapid Test Cassette (Swab) is limited to laboratory personnel and CLIA waived users. Not for home use.
- This device is a qualitative test and does not provide information on the viral load present in the specimen.
- This test detects both viable (live) and non-viable influenza A, influenza B, and SARS-CoV-2. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the sample.
- A negative test result may occur if the level of antigen in the sample is below the detection limit of the test or if the sample is collected, handled or transported incorrectly.
- Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
- Exposure to hand sanitizer may cause false negative results with this test.
- Individuals who recently received nasally administered influenza A or influenza B vaccine may have false positive test results after vaccination.

QUALITY CONTROL

Internal Quality Control

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient sample volume, test functionality and correct procedural technique.

External Quality Control

Positive and negative controls should be tested as good laboratory practice to confirm the test procedure and to verify test performance. Quality control testing should be performed according to:

- Local, state, and/or federal regulations.
- Your laboratories' quality control procedures.

Control materials are not supplied with this kit and can be purchased separately. Healgen® offers External Controls - Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) (Catalog Number: GCFC-PN2, GCFC-PN20). It is recommended to run external controls once-

- every 30 days (to check storage)
- each new shipment
- each new kit lot
- each new operator
- as required by site quality control procedures and in accordance with local, state, and federal regulations or any accreditation requirements.

This will verify that the reagents and test cassettes are working properly, and that the operator is able to perform the test procedure correctly.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY AND PATIENT CARE SETTINGS

The Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical- devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

However, to assist in using the Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) ("your product" in the conditions below). the relevant Conditions of Authorization are listed below:

• Authorized laboratories* using your product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating this labeling may be used, which may include mass media.

• Authorized laboratories using your product must use your product as outlined in Healgen® COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) Instructions for Use and Quick Reference Guide. Deviations from the authorized procedures, including authorized instruments, authorized clinical specimen types, authorized control materials, authorized ancillary reagents and authorized materials required to use your product are not permitted.

• Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

• Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
• **Authorized laboratories must collect information on the performance of your product and report any significant deviations from the established performance characteristics of your product of which they become aware to DMD/OHT7-OIR/OPEQ/CDRH** (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Healgen Scientific, LLC by contacting Technical Services (via email at Support@Healgen.com or via phone at 866-982-3818).
• All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
• **Healgen Scientific, LLC, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.**

*The Letter of Authorization refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate complexity, high complexity, or waived tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation" as "Authorized Laboratories".

PERFORMANCE CHARACTERISTICS

Clinical Performance

A prospective study was completed at ten sites in the United States for clinical validation of the Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) for the detection of the SARS-CoV-2/Flu A/Flu B in self-collected anterior nasal (AN) swab samples. The study evaluated the Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) performance in symptomatic individuals who were currently experiencing symptoms associated with COVID-19, influenza A and/or influenza B. A total of 1122 subjects experiencing symptoms associated with COVID-19/Flu A/Flu B were enrolled in the study. 1122 were evaluable, of which 1122 subjects were evaluable for Flu A/B, and 1097 were evaluable for SARS-CoV-2.

Each enrolled subject either self-collected a dual anterior nares (AN) sample or had a dual AN sample collected from him/her by another individual for the investigation test. Each subject also had a dual AN sample collected from him/ her by one of the study personnel for the comparator tests, which were FDA cleared RT-PCR assays. Swab collections for investigation and comparator samples were alternated by subject. The comparator tests were performed according to their respective instructions for use. Test results from the Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) were compared to the results generated from comparator tests. Results are shown in Tables 1.1-1.3.

Table 1.1: Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) Test Results for SARS-CoV-2 versus RT-PCR Comparator (Based on 5 DPSO)

	Comparator		
Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) SARS-CoV-2 Test Results	**Positives**	**Negatives**	**Total**
Positives	69	10	**79**
Negatives	6	1012	**1018**
Total	**75**	**1022**	**1097**

Positive Percent Agreement = (69/75) = 92.0% (95% CI: 83.6% - 96.3%)
Negative Percent Agreement = (1012/1022) = 99.0% (95% CI: 98.2% - 99.5%)

Table 1.2: Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) Test results for FLU A versus RT-PCR Comparator (Based on 5 DPSO)

	Comparator		
Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) FLU A Test Results	**Positives**	**Negatives**	**Total**
Positives	49	1	**50**
Negatives	4	1068	**1072**
Total	**53**	**1069**	**1122**

Positive Percent Agreement = (49/53) = 92.5% (95% CI: 82.1% - 97.0%)
Negative Percent Agreement = (1068/1069) = 99.9% (95% CI: 99.5% - 100.0%)

Table 1.3: Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) FLU B versus RT-PCR Comparator

	Comparator		
Healgen® COVID-19/Flu A&B Ag Combo Rapid test Cassette (Swab) FLU B Test Results	**Positives**	**Negatives**	**Total**
Positives	38	1	**39**
Negatives	4	1079	**1083**
Total	**42**	**1080**	**1122**

Positive Percent Agreement = (38/42) = 90.5% (95% CI: 77.9% - 96.2%)
Negative Percent Agreement = (1079/1080) = 99.9% (95% CI: 99.5% - 100.0%)

Clinical Performance in Subjects on Different Symptomatic Days for SARS-CoV-2

Table 2: SARS-CoV-2 Clinical Performance on Days Post Symptoms Onset (Based on 5 DPSO)

Days post-COVID-19 Symptoms Onset	Number of Subject samples tested	Positives	Comparator Positives	% Positive Rate (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%)**

Subject Demographics

Table 3: Subject Demographics of All Enrollments

Demographic	Subjects (by lay-user collection and testing (N=178)	Self-collecting and testing (N=944)	Overall (N=1122)
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]
Age Group			
⩾2 - <14 years of age	171 (96.1%)	0 (0.0%)	171 (15.2%)
14 - 24 years of age	6 (3.4%)	166 (17.6%)	172 (15.3%)
>24 - 64 years of age	0 (0.0%)	691 (73.2%)	691 (61.6%)
⩾65 years of age	1 (0.6%)	87 (9.2%)	88 (7.8%)
Total	178 (100.1%)	944 (100.0%)	1122 (99.9%)
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			
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%)
Total	**178 (100.0%)**	**944 (100.0%)**	**1122 (100.0%)**

SERIAL TESTING

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States.

Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 – 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RTPCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection.

Performance of the antigen test with serial testing in symptomatic individuals is described in the table below. Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator

single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

Day After First PCR Positive Test Result	Symptomatic on Frist Day of Testing		
	AG Positive / PCR Positive (Antigen Test Performance %PPA)		
	1 Test	2 Test	3 Test
0	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	58/62 (93.5%)	59/60 (98.3%)	43/43 (100.0%)
4	55/58 (94.8%)	53/53 (98.1%)	39/40 (97.5%)
6	27/34 (79.4%)	26/33 (79.8%)	22/27 (81.5%)
8	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	4/9 (44.4%)	3/7 (42.9%)	-

1 Test = one (1) test performance on the noted days after first PCR positive test result. Day 0 is the first day of documented infection with SARS-CoV-2.

2 Tests = two (2) tests performance an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.

3 Tests = three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

Analytical Performance

Analytical Sensitivity: Limit of Detection (LoD)

The limit of detection (LoD) Was established using dilutions of one SARS-Related Coronavirus 2 (SARS-CoV-2) (USA-WA1/2020), two influenza A strains (H1N1pdm09: A/Victoria/4897/2022, H3N2: A/Darwin/6/2021) and two Influenza B strains (Victoria: B/Washington/02/2019, Yamagata: B/Florida/4/2006) in negative clinical matrix. The isolate dilutions were tested by adding fifty (50) µL to the head of the nasal swab and extracting the swab per the Healgen COVID-19/Flu A&B Ag Combo Rapid Test Cassette (Swab) Instructions for Use.

In this study, range finding testing was followed by final dilution testing to determine the LoD of the assay. Range finding involved testing a series of 10-fold dilutions in replicates of three (3) to determine the starting point for the dilution series to determine LoD. The dilution of each virus which resulted in the lowest concentration that generated 100% positive detection rate was set as the target for the next dilution series, which involved testing twenty (20) replicates of two (2)-fold dilutions. In the final dilution testing, the lowest concentration that generated ≥95% positive detection rate was set as the LoD concentration. Confirmatory testing was done on 3 lots totaling sixty (60) replicates.

The LoD for the analytes is identical when analytes are co-spiked into the same sample. The results of LoD confirmation testing for each virus are summarized in Table 3a. The LoD studies with the WHO standard are determined similarly and the results are included in Table 3b.

Table 3a: Confirmatory LoD Determination

Analyte	Lineage	Virus Strains	LoD Concentration in PNSM ($TCID_{50}$/mL)	Concentration on swab ($TCID_{50}$/ mL)	#Positives/# Tested
SARS-CoV-2	N/A	USA-WA1/2020	3.95E+02	1.98E+01	60/60
Flu A	H3N2	A/Darwin/6/2021	2.09E+02	1.04E+01	60/60
	H1N1	A/Victoria/4897/22	2.02E+02	1.01E+01	60/60
Flu B	Yamagata	B/Florida/4/2006	1.46E+01	7.31E-01	60/60
	Victoria	B/Washington/02/2019	1.58E+03	7.90E+01	60/60

Table 3b: WHO SARS-Cov2 Standard Antigen LoD

Description	Source	NIBSC. No.	Concentration (IU/ mL)	IU/Swab	#Positives/ # Tested
WHO International	NIBSC	21/368	250	12.5	20/20

Hook Effect

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 the study, the highest concentration possible of UV inactivated SARS-CoV-2 virus stock, each of the live Influenza A virus stock, H1N1 pdm09 and H3N2, and each live Influenza B virus stock, Victoria and Yamagata, were spiked onto the sterile swab and tested in triplicate on the Healgen® multiplex test to test for a high-dose hook effect.

The Healgen® multiplex test showed no hook effect for SARS-CoV-2, Flu A, and Flu B, at the concentrations listed in Table 4.

Table 4: Summary of Hook Effect

Virus	Subtype or Lineage	Concentration without Hook Effect ($TCID_{50}$/mL)
SARS-CoV-2	NA	3.16E+06
Influenza A	H1N1	2.02E+05
Influenza A	H3N2	4.17E+05
Influenza B	Victoria	3.16E+06
Influenza B	Yamagata	1.17E+05

Analytical Specificity: Cross Reactivity (Exclusivity) and Microbial Interference

The Healgen® COVID-19/Flu A&B Combo Rapid Test Cassette (Swab) showed no cross-reactivity and no microbial interference for the listed organisms when tested in the concentrations listed in Table5.

Table 5: Summary of Cross-reactivity and Microbial Interference

ID	Organism	Concentration tested	Units	Cross-reactivity	Microbial Interference
SARS	SARS-CoV-1	1.25E+05	PFU/mL	ND*	ND
MERS	MERS-coronavirus	1.47E+05	$TCID_{50}$/mL	ND	ND
OC43	Human coronavirus OC43	7.00E+05	$TCID_{50}$/mL	ND	ND
229E	Human coronavirus 229E	1.58E+05	$TCID_{50}$/mL	ND	ND
NL63	Human coronavirus NL63	8.00E+04	$TCID_{50}$/mL	ND	ND
AV1	Adenovirus, Type 1 (Adenoid 71)	2.23E+05	$TCID_{50}$/mL	ND	ND
AV7	Adenovirus Type 7, Type 7A (Species B)	1.58E+05	$TCID_{50}$/mL	ND	ND
CMV	Cytomegalovirus, Strain AD-169	7.05E+04	$TCID_{50}$/mL	ND	ND
EBV	Epstein Barr Virus, Strain B95-8	1.83E+06	CP/mL	ND	ND
hMPV	Human Metapneumovirus (hMPV), Strain TN/91-316	3.50E+05	$TCID_{50}$/mL	ND	ND
P1	Parainfluenza virus 1, Strain	2.00E+05	$TCID_{50}$/mL	ND	ND
P2	Parainfluenza virus 2, Strain Greer	1.75E+05	$TCID_{50}$/mL	ND	ND
P3	Parainfluenza virus 3, Strain C243	7.00E+05	$TCID_{50}$/mL	ND	ND
P4	Parainfluenza virus 4, Strain N/A	2.39E+05	$TCID_{50}$/mL	ND	ND
EV68	Enterovirus Type (e.g. 68), Species D Type 68	2.23E+05	$TCID_{50}$/mL	ND	ND
RSVA	Respiratory syncytial virus A, Strain A-2	3.50E+05	$TCID_{50}$/mL	ND	ND
RSVB	Respiratory syncytial virus B, Strain CH93(18)-18	2.29E+05	$TCID_{50}$/mL	ND	ND
RV	Rhinovirus 1A, Strain N/A	7.05E+04	$TCID_{50}$/mL	ND	ND
BP	Bordetella pertussis, Strain A639	2.50E+08	CFU/mL	ND	ND
CA	Candida albicans, Strain Z006	6.03E+06	CFU/mL	ND	ND
CP	Chlamydia pneumoniae, Strain Z500	4.33E+06	IFU/mL	ND	ND
CB	Corynebacterium xerosis	2.30E+07	CFU/mL	ND	ND
EC	Escherichia coli, Strain mcr-1	1.79E+08	CFU/mL	ND	ND
HI	Hemophilus influenzae, type b; Eagan	9.68E+06	CFU/mL	ND	ND
LB	Lactobacillus sp., Lactobacillus Acidophilus, Strain Z048	1.21E+07	CFU/mL	ND	ND
LP	Legionella spp pneumophila, Strain Philadelphia-1	6.50E+06	CFU/mL	ND	ND
MC	Moraxella catarrhalis, Strain 59632	2.50E+08	CFU/mL	ND	ND
MP	Mycoplasma pneumoniae, Strain PI 1428	2.50E+07	CFU/mL	ND	ND
MT	Mycobacterium tuberculosis avirulent, Strain	4.15E+06	CFU/mL	ND	ND
NM	Neisseria meningitidis, serogroup A	3.43E+06	CFU/mL	ND	ND
NS	Neisseria sp. Elongata Z071	2.68E+08	CFU/mL	ND	ND
PJ	Pneumocystis jirovecii, Strain W303-Pji	1.30E+07	CFU/mL	ND	ND
PA	Pseudomonas aeruginosa, Strain N/A	3.45E+08	CFU/mL	ND	ND
SA	Staphylococcus aureus Protein A producer, e.g., Cowan strain, NCTC 8530 [S11]; Cowan's serotype 1	2.60E+08	CFU/mL	ND	ND
SE	Staphylococcus epidermidis (PCI 1200)	9.00E+07	CFU/mL	ND	ND
SS	Streptococcus salivarius, Strain C699 [S30D]	1.01E+06	CFU/mL	ND	ND
SPN	Streptococcus pneumoniae, Strain Z022	1.81E+07	CFU/mL	ND	ND
SPY	Streptococcus pyogenes, Strain MGAS 8232	7.50E+07	CFU/mL	ND	ND
ME	Measles, Strain Edmonston	8.48E+05	$TCID_{50}$/mL	ND	ND
MU	Mumps (Isolate 1)	8.48E+05	$TCID_{50}$/mL	ND	ND

**ND: Not Detected.*

Competitive Interference

The Healgen® COVID-19/Flu A&B Combo Rapid Test Cassette (Swab) test showed no competitive interference from the analytes co-existed in the specimens at the concentrations indicated in Table 6.

Table 6: Competitive Interference Tolerated

Analyte	Lineage	LoD Concentration in PNSM	Competitive Interference Tolerated ($TCID_{50}$/mL)	
SARS- CoV-2	N/A	3.95E+02	2666.7X LoD	1.05E+06
Flu A	H3N2: A/Darwin/6/2021	6.26E+02	666.7X LoD	1.39E+05
Flu B	(Yamagata) B/Florida/4/2006	1.46E+01	2666.7X LoD	3.90E+04

Inclusivity (In silico & Analytical Sensitivity)

The Healgen® COVID-19/Flu A&B Combo Rapid Test Cassette (Swab) test shows inclusivity detection for virus variants similar to SARS-COV-2, Flu A, and Flu B at the listed and higher concentrations in Table 7.

Table 7: Inclusivity Summary – Lowest Concentrations Tested Positive for Relevant Virus Strains

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	4.17E+03	$TCID_{50}$/mL
	A/ NY/03/2009	2.29E+04	$TCID_{50}$/mL
	A/ Indiana/02/2020	2.43E+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
Flu A – H3N2	A/New York/21/2020	2.6E+05	FFU/mL
	A/Tasmania/503/2020	6.5E+04	FFU/mL
	A/Hong Kong/2671/2019	1.6E+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
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	TCID50/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

Interfering Substances

The Healgen® COVID-19/Flu A&B Combo Rapid Test Cassette (Swab) test showed no interference from the potential substances at the concentrations indicated in Table 8.

Table 8 Potential Interfering Substances

Potential Interfering Substances	Concentration Tolerated
Human Whole Blood (EDTA tube)	4% v/v
Mucin	0.50%
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
Nasal Drops (Phenylephrine)	15% v/v
Nasal Spray (Oxymetazoline)	15% v/v
Nasal Spray (Cromolyn)	15% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	10% v/v
Sore Throat Phenol Spray	15% v/v
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL
FluMist®/ FluMist® Quadrivalent- Live intranasal influenza virus vaccine	0.15% v/v
Zanamivir	282 ng/mL
Biotin	3,500 ng/mL
Body & Hand Lotion	0.5% w/v
Body Lotion, with 1.2% dimethicone	0.5% w/v
Hand Lotion	5% w/v
Hand Sanitizer with Aloe, 62% ethyl alcohol	5% v/v
Hand Sanitizer cream lotion	7.5% w/v
Hand Sanitizer, 80% ethanol, fast drying	7.5% w/v
Hand soap liquid gel	0.05% w/v

Precision

A single-site precision study was conducted to determine between-lot, between-operator, between-run, between-day and total precision calculations. Two (2) trained operators tested three (3) lots of reagents/strips/cassettes across ten (10) days. Lots were differentiated by different batches of raw materials (i.e., lots that do not derive from the same in-process batches of raw materials and components, including multiple raw material lots for critical reagents such as antibodies). Samples were prepared, randomized, and blinded before testing. Three sample levels were tested each day (2X single analyte LoD co-spiked, 5X single analyte LoD co-spiked, and Negative, contrived in the pooled negative nasal wash) one (1) replicate per run, per operator, per lot. Two (2) runs (at least 4 hours apart or morning and afternoon) were conducted each day per operator, per lot, per day. This exact testing schema 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. The results are shown in Table 9.

Table 9: Summary of Precision Results

Sample	Analyte	N	Between Lot agreement STD DEV (%)	Between Day agreement STD DEV (%)	Between Operator agreement STD DEV (%)	Between Run agreement STD DEV (%)	Total agreement STD DEV (%)
Negative	SARS-CoV-2	120	0	0	0	0	0
	Flu A	120	0	0	0	0	0
	Flu B	120	0	0	0	0	0
2X LoD	SARS-CoV-2	120	0	0	0	0	0
	Flu A	120	0	0	0	0	0
	Flu B	120	0	0	0	0	0
5X LoD	SARS-CoV-2	120	0	0	0	0	0
	Flu A	120	0	0	0	0	0
	Flu B	120	0	0	0	0	0

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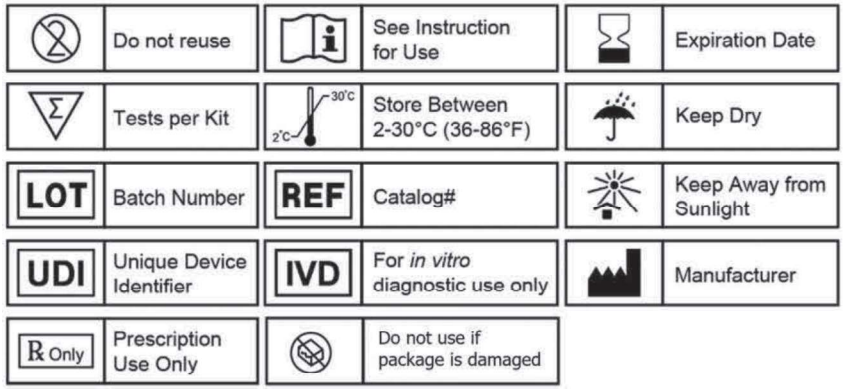
INDEX OF SYMBOLS



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