Version 4.0
Update: 11/06/2020

cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit

Instructions for use

REF : L00847	96 Tests
REF : L00847-5	480 Tests

For *In Vitro* Diagnostic Use Under FDA Emergency Use Authorization

For Prescription Use Only

The operator should read technical manual carefully before using this product.

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Contents

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I. INTENDED USE

The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is a Blocking Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative direct detection of total neutralizing antibodies to SARS-CoV-2 in human serum and K_2-EDTA plasma. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit should not be used to diagnose acute SARS-CoV-2 infection.

At this time, it is unknown for how long antibodies persist following infection and if the presence of neutralizing antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a to perform high complexity tests.

Results are for the detection of SARS CoV-2 total neutralizing antibodies. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time neutralizing antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

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

The sensitivity of the cPass Neutralization Antibody Detection kit early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

II. BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, or 2019-nCoV) is an

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enveloped non-segmented positive-sense RNA virus. It is the causative agent of coronavirus disease 2019 (COVID-19), which is contagious in humans.

SARS-CoV-2 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) contains a receptor-binding domain (RBD), which is responsible for recognizing the cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication.

Infection with SARS-CoV-2 initiates an immune response, which includes the production of antibodies, or binding antibodies, in the blood. Not all binding antibodies can block cellular infiltration and replication of the SARS-CoV-2 virus. The subpopulation of the binding antibodies that can block cellular infiltration and replication of the virus are named neutralizing antibodies. It is unknown how long it takes for neutralizing antibodies to be produced, and if they are always produced after SARS-CoV-2 infection. While individuals infected with SARS-CoV-2 develop binding antibodies to the virus, not all of them develop neutralizing antibodies to SARS-CoV-2. The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is specific to SARS-CoV-2 neutralizing antibodies.

III. ASSAY PRINCIPLE

The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is a blocking ELISA detection tool. Using purified receptor binding domain (RBD), protein from the viral spike (S) protein and the host cell receptor ACE2, this test is designed to mimic the virus-host interaction by direct protein-protein interaction in a test tube or a well of an ELISA plate. The highly specific interaction can then be neutralized, the same manner as in a conventional Virus Neutralization Test (VNT). Recent studies demonstrated that the cPass test does not detect binding antibodies such as those typically detected in conventional indirect ELISA tests, but rather the presence of neutralizing antibodies.

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The kit contains two key components: the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD) and the human ACE2 receptor protein (hACE2). The protein-protein interaction between HRP-RBD and hACE2 can be blocked by neutralizing antibodies against SARS-CoV-2 RBD.

Samples and controls diluted with sample dilution buffer and pre-incubated with the HRP-RBD to allow the binding of the circulating neutralization antibodies to HRP-RBD. The mixture is then added to the capture plate, which is pre-coated with the hACE2 protein. The unbound HRP-RBD as well as any HRP-RBD bound to non-neutralizing antibody will be captured on the plate, while the circulating neutralization antibodies HRP-RBD complexes remain in the supernatant and are removed during washing. Following a wash cycle, TMB substrate solution is added followed by the Stop Solution and the reaction is then quenched and the color turns yellow. The absorbance of the final solution can be read at 450 nm in a microtiter plate reader.

IV. KIT CONTENTS

Component	96 Tests		480 Tests	
	Quantity	REF	Quantity	REF
Capture Plate*	1 plate	S1-80	5 plates	S5-80
Positive Control	1 vial (0.05 mL)	S1-10	1 vial (0.25 mL)	S5-10
Negative Control	1 vial (0.05 mL)	S1-11	1 vial (0.25 mL)	S5-11
HRP conjugated RBD	1 vial (0.02 mL)	S1-30	1 vial (0.1 mL)	S5-30
HRP Dilution Buffer	1 bottle (10 mL)	S1-90	1 bottle (50 mL)	S5-90
Sample Dilution Buffer	1 bottle (30 mL)	S1-60	1 bottle (150 mL)	S5-60
20× Wash Solution	1 bottle (40 mL)	S1-70	1 bottle (200 mL)	S5-70

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TMB Solution	1 bottle (12 mL)	S1-40	1 bottle (60 mL)	S5-40
Stop Solution	1 bottle (6 mL)	S1-50	1 bottle (30 mL)	S5-50
Plate Sealer	2 pieces	N/A	10 pieces	N/A

*Capture Plate: Pre-coated 96 well microplates (8 wells x 12 strips); 12 strips configured in plate sealed in a foil pouch with a desiccant.

V. STORAGE

The unopened kit is stable for 6 months from the date of manufacture if stored at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

VI. WARNINGS

For Prescription and *In Vitro* Diagnostic Use only

1. For Use under an Emergency Use Authorization Only
2. This test has not been FDA cleared or approved;
3. This test has been authorized by FDA under an EUA for use by authorized laboratories;
4. This test has been authorized only for the presence of total neutralizing antibodies against SARS-CoV-2, not for any other viruses or pathogens; and
5. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
6. Human source material used to prepare the controls included in this kit should be handled as potentially infectious material. Use universal precautions when handling.
7. Do not pipette by mouth.
8. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
9. Wear disposable gloves while handling the kit reagents and wash hands thoroughly

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afterwards.

10. Certain components of this product contain 0.03% ProClin 300 as a preservative, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals
11. Certain components are labeled with the following:

Irritating to eyes (R 36). Irritating to skin (R 38). Avoid contact with skin (S 24). Avoid contact with eyes (S 25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). Wear suitable protective clothing (S 36). If swallowed, seek medical advice immediately and show this container or label (S 46).

VII. PRECAUTIONS

1. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents should be handled at the Biosafety Level 2.
2. Do not mix components from different batches. Do not mix with components from other manufacturers.
3. Do not use reagents beyond the stated expiration date.
4. All reagents must return to room temperature (20°C to 25°C) before running assay. Use the required volume of reagents only. Do not pour reagents back into vials as reagent contamination may occur.
5. Before opening Positive Control and Negative Control, tap the vial on the benchtop to ensure that all liquid is at the bottom of the vial.
6. Use only distilled or deionized water and clean glassware.
7. Do not let wells dry during test; add reagents immediately after washing steps.

VIII. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Single or dual wavelength microplate reader with 450 nm filter. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

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- Automated microplate washer to wash the plate
- Deionized or distilled water to dilute 20× Wash Solution
- Graduated cylinder to prepare Wash Solution
- Plastic container to store Wash Solution
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL and 1000 µL precision pipettes
- 10 µL, 200 µL and 1000 µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Paper towel
- Laboratory timer
- Refrigerator to store samples and kit components
- Centrifuge
- 37 ºC Incubator

IX. SPECIMEN COLLECTION AND STORAGE

1. Handle all serum and K_2-EDTA plasma as if capable of transmitting infectious agents.
2. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).
3. For performance of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, a minimum volume of 50 µL per serum or K_2-EDTA plasma sample is recommended, in case that repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
4. For human serum, use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000 g. Run assay immediately, otherwise store aliquot below -20°C. Avoid repeated freeze-thaw cycles.

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5. For human plasma, treat blood with the anticoagulant K_2-EDTA. Centrifuge for 10 minutes at 1000 g within 30 minutes for plasma collection. Run assay immediately, otherwise store samples below -20°C. Avoid repeated freeze-thaw cycles.

X. PROTOCOL

■ Reagent Preparation

1. All reagents must be taken out from refrigeration and allowed to return to room temperature before use (20° to 25°C). Save all reagents in refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. HRP-RBD Preparation: Dilute HRP conjugated RBD with a 1:1000 dilution ratio with RBD Dilution Buffer. For example, dilute 10 µL of HRP conjugated RBD with 10 mL of HRP Dilution Buffer to make a HRP-RBD solution.
4. 1× Wash Solution Preparation: Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2°C to 8°C.

Note: If any precipitate is observed in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.

■ Sample and Control Dilution

Dilute test samples, Positive, and Negative Controls with Sample Dilution Buffer with a volume ratio of 1:9. For example, dilute 10 µL of sample with 90 µL of Sample Dilution Buffer.

■ Capture Plate Preparation

1. It is recommended that all Positive Controls, Negative Controls, and samples should be prepared in duplicate.
2. Count the ELISA strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.

Test Configuration

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	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative Control											
B	Negative Control											
C	Positive Control											
D	Positive Control											
E												
F												
G												
H												

3. Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be stored in the closed foil pouch to prevent moisture damaging the Capture Plate.

■ Test Procedure

Neutralization Reaction

1. In separate tubes, mix the diluted Positive Control, diluted Negative Control, and the diluted samples with the diluted HRP-RBD solution with a volume ratio of 1:1. For example, mix 60 µL Positive Control with 60 µL HRP-RBD solution. Incubate the mixtures at 37°C for 30 minutes.
2. Add 100 µL of each of the positive control mixture, the negative control mixture, and the sample mixture to the corresponding wells.
3. Cover the plate with Plate Sealer and incubate at 37°C for 15 minutes.
4. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
5. Tap the plate on paper towel to remove residual liquid in the wells after washing steps.

Substrate Reaction and Absorbance Measurement

6. Add 100 µL of TMB Solution to each well and incubate the plate in dark at 20 - 25°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
7. Add 50 µL of Stop Solution to each well to stop the reaction.
8. Read the absorbance in microtiter plate reader at 450 nm immediately.

Note: The substrate reaction time is determined by the temperature, the ideal reaction temperature is 25°C.

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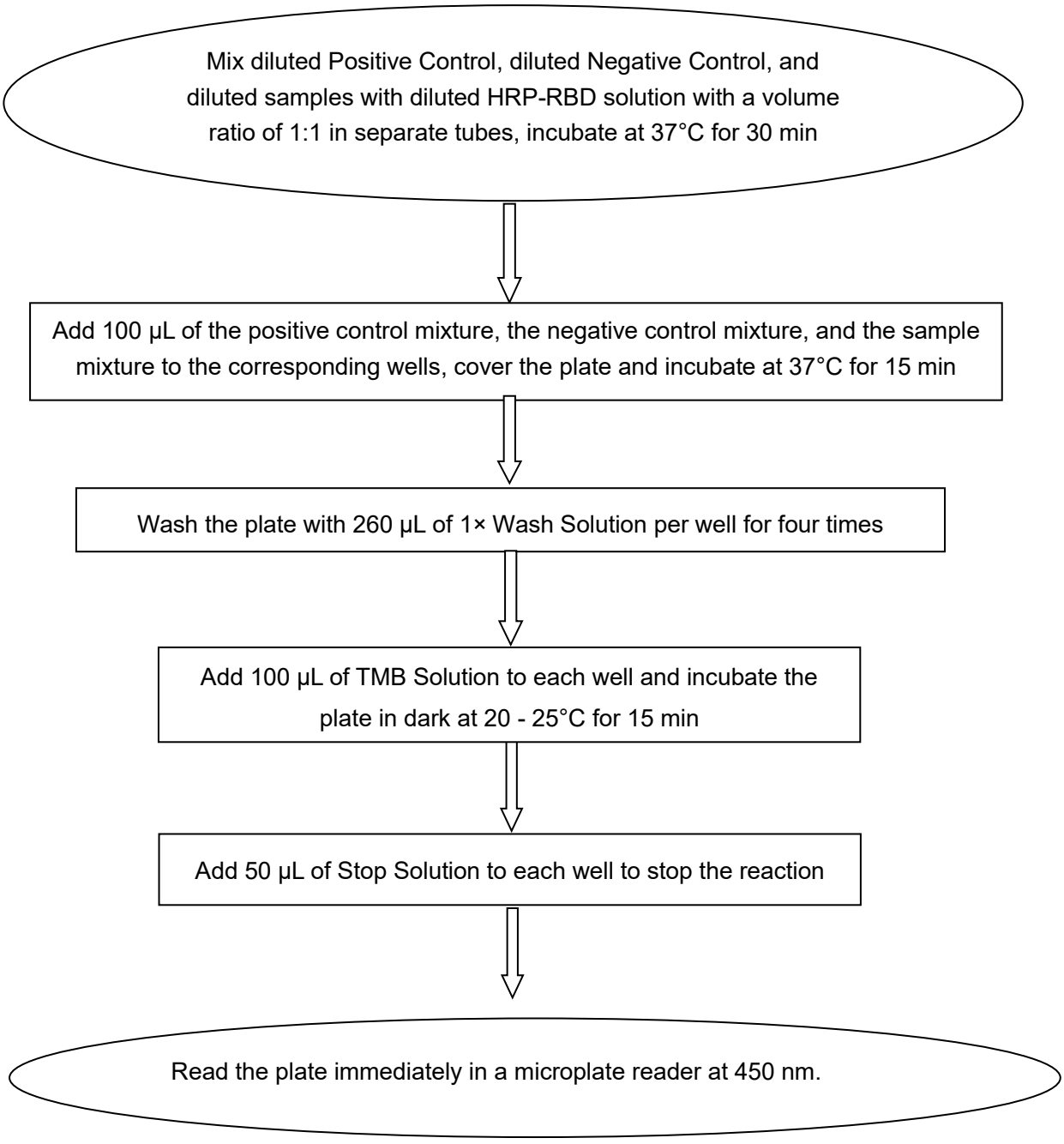
XI. ASSAY PROCEDURE SUMMARY



XII. QUALITY CONTROL

To assure the validity of the results, each assay must include both Positive and Negative Controls. The average optical density (OD450) of each control must fall within the values listed in the following table. If OD450 values of controls do not meet the requirements in the following table, the test is invalid and must be repeated.

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- OD450 values for quality control

Items	OD450 value	Control Result for Valid Assay
Quality Control	＞ 1.0	Negative Control
	＜ 0.3	Positive Control

Note：The standards in the table are only intended to evaluate the performance of the kit.

Additionally, the coefficient of variation (CV) of OD signal values determined for all replicates of positive and negative samples must be ≤10% within the same run.

XIII. INTERPRETION OF RESULTS

The 30% signal inhibition cutoff for SARS-CoV-2 neutralizing antibody detection can be used for interpretation of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit signal inhibition rate. The operator can determine the result of the sample by comparing the inhibition rate to the following table.

$$\text{Percent Signal Inhibition} = \left(1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}}\right) \times 100\%$$

XIV. Result Interpretation

Value Results*	Result	Test Result Interpretation
≥ 30% Signal Inhibition	Positive	Neutralizing antibodies for SARS-CoV-2 are detected **
＜ 30% Signal Inhibition	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected.**

*Value Results observed with the cPass SARS-CoV-2 Neutralization Antibody Detection Kit are not reported to the end user since this test is for qualitative use.

** The cPass SARS-CoV-2 Neutralization Antibody Detection Kit results have shown 100% positive percent agreement (95% CI 87.1-100.0%) and 100% negative percent agreement 100.0% (95% CI 95.8-100.0%) with $PRNT_{50}$ and $PRNT_{90}$ in clinical study.

The clinical applicability of detection or correlation with neutralizing activity for total antibodies to the SARS-CoV-2 receptor binding domain (RBD) at 50% and 90% viral neutralization is currently unknown and results cannot be interpreted as an indication of degree of immunity or protection from reinfection. Because SARS-CoV-2 neutralizing

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antibody assays are not standardized, and the performance characteristics of each SARS-CoV-2 neutralizing antibody test is uniquely established, results from different SARS-COV-2 neutralizing antibody assays are not comparable.

XV. LIMITATIONS OF THE PROCEDURE

- This test is designed for qualitative detection of SARS-CoV-2 neutralizing antibodies.
- To be used only under the conditions of the FDA Emergency Use Authorization.
- Negative results do not rule out SARS-COV-2 infection, particularly those who have been in contact with the virus. Direct testing with a molecular diagnostic should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.
- Positive results may be due to current or past infection with non-SARS-COV-2 corona virus strains, such as HKU1, NL63, OC43, or 229E.
- Results from this test should not be used to diagnose or to exclude acute SARS-COV-2 infection or to inform infection status.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
- The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is known to cross-react with SARS-CoV-1 neutralizing antibodies.
- This test should not be used for blood donor screening.

CONDITIONS OF AUTHORIZATIONS FOR THE LABORATORIES:

The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit 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.

Authorized laboratories using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (“your product” in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

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

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B. Authorized laboratories using your product will use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

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

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Genscript USA Inc (at qa@genscript.com) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product.

G. Genscript USA Inc, Nanjing GenScript Biotech Co Ltd, authorized distributors, and authorized laboratories using your product will 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, to perform high complexity tests” as “authorized laboratories.”

XVI. Potentially Cross-Reactivity

To evaluate the potential cross-reactivity of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit, positive and negative controls and 60 clinical specimen seropositive for other diseases were tested in duplicate. The table below summarizes the results with the cPass SARS-CoV-2 Neutralization Antibody Detection Kit table for wet tested organisms:

Samples	Seropositive for Disease	cPass SARS-CoV-2 Neutralization Antibody Detection Kit		
		%CV	Mean Value Result (% signal inhibition)	Result Reported (Positive ≥ 30%)
1	Influenza A	1%	8%	Negative

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2	Influenza A	3%	5%	Negative
3	Influenza A	0%	-9%	Negative
4	Influenza A	2%	1%	Negative
5	Influenza A	3%	5%	Negative
6	Influenza A/B IgM	3%	-1%	Negative
7	Influenza A IgG	4%	-7%	Negative
8	Influenza B IgG	4%	7%	Negative
9	Influenza A IgG	7%	1%	Negative
10	Influenza A/B IgM	4%	-8%	Negative
11	Influenza B IgG	2%	2%	Negative
12	HCV	1%	4%	Negative
13	HCV	1%	3%	Negative
14	HCV	5%	-1%	Negative
15	HCV	4%	0%	Negative
16	HCV	1%	4%	Negative
17	ANA	1%	12%	Negative
18	ANA	4%	-2%	Negative
19	ANA	3%	-11%	Negative
20	ANA	1%	-1%	Negative
21	ANA	3%	3%	Negative
22	RSV IgG	2%	-7%	Negative
23	RSV IgG	4%	-10%	Negative
24	RSV IgG	1%	-4%	Negative
25	HBsAB	3%	-4%	Negative
26	HBsAB	0%	-8%	Negative
27	HBsAB	1%	-9%	Negative
28	HBsAB	2%	-3%	Negative
29	HBc IgM	4%	-1%	Negative
30	HBc IgM	0%	-6%	Negative
31	HBc IgM	3%	-4%	Negative
32	HBc IgM	1%	-1%	Negative
33	HBc IgM	8%	-4%	Negative
34	HBsAB	3%	-11%	Negative
35	RSV IgG	0%	-2%	Negative
36	RSV IgM	2%	-5%	Negative
37	RSV IgM	1%	-5%	Negative
38	RSV IgM	3%	-10%	Negative
39	HIV	7%	16%	Negative
40	HIV	10%	7%	Negative
41	HIV	4%	6%	Negative
42	HIV	7%	7%	Negative
43	HIV	0%	19%	Negative
44	HIV	3%	13%	Negative

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45	HIV	6%	10%	Negative
46	HIV	4%	13%	Negative
47	HIV	2%	17%	Negative
48	HIV	9%	12%	Negative
49	hCoV 229E	0%	10%	Negative
50	hCoV 229E	0%	10%	Negative
51	hCoV OC43	2%	11%	Negative
52	hCoV OC43	12%	9%	Negative
53	SARS-CoV-1*	4%	36%	Positive
54	SARS-CoV-1*	8%	59%	Positive
55	MERS-CoV	5%	12%	Negative
56	MERS-CoV	3%	12%	Negative
57	Dengue	4%	-1%	Negative
58	Dengue	3%	2%	Negative
59	Dengue	4%	1%	Negative
60	Zika	1%	7%	Negative

* The results show cross-reactivity to anti-SARS-CoV-1 positive samples. No cross-reactivity was observed with any of the hCoV sera tested nor any of the other anti-sera tested in this study.

XVII. CLINICAL PERFORMANCE

In order to validate the clinical performance of the GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit, the comparator Plaque Reduction Neutralization Test (PRNT) utilizing the SARS-CoV-2 virus (WA01/2020 isolate) was used. The cutoff for the PRNT comparator tests was established as indicated below:

$PRNT_{50}$:

Value Result (dilution titer)	**Result**	**Test Result Interpretation**
≥ 1:20	Positive	Neutralizing antibodies for SARS-CoV-2 are detected at 50% viral neutralization.
≤ 1:20	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected at 50% viral neutralization.

$PRNT_{90}$:

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Value Result (dilution titer)	Result	Test Result Interpretation
≥ 1:10	Positive	Neutralizing antibodies for SARS-CoV-2 are detected at 90% viral neutralization.
≤ 1:10	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected at 90% viral neutralization.

The clinical agreement study evaluated a total of 114 samples retrospectively collected from SARS-CoV-2 RT-PCR positive and negative individuals (26 PRNT positive and 88 PRNT negative) using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit and the PRNT comparator ($PRNT_{50}$ and $PRNT_{90}$). The combined cohort consisted of samples from normal healthy people (n=88) and samples from RT-PCR confirmed SARS-CoV-2 positive patients (n=26). The GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit sample results were compared to a Plaque Reduction Neutralization Test performed to WHO guidelines. The following tables show the Positive and Negative Percent Agreement between the $PRNT_{50}$ or $PRNT_{90}$ and the cPass SARS-CoV-2 Neutralization Antibody Detection Kit result.

Clinical Agreement using $PRNT_{50}$ titers as the comparator method

		Plaque Reduction Neutralization Test ($PRNT_{50}$)	
		Positive (n=26)	Negative (n=88)
GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit	Positive	26	0
	Negative	0	88
	Positive Percent Agreement	100% (95% CI 87.1-100.0%)	
	Negative Percent Agreement		100.0% (95% CI 95.8-100.0%)

Clinical Agreement using $PRNT_{90}$ titers as the comparator method

		Plaque Reduction Neutralization Test ($PRNT_{90}$)	
		Positive (n=26)	Negative (n=88)
GenScript cPass SARS-CoV-2 Neutralization Antibody	Positive	26	0
	Negative	0	88
	Positive Percent Agreement	100% (95% CI 87.1-100.0%)	
	Negative Percent Agreement		100.0%

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Detection Kit			(95% CI 95.8-100.0%)

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XIX. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Weak/No Signal	Substrate are not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	Substrate are contaminated	Use new Substrate with same Lot
	Volumes of reagents are not correct	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	The plate is not read immediately	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	Substrate is contaminated	Use new substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with plate sealer in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay

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