Wantai SARS-CoV-2 Diagnostics

WANTAI SARS-CoV-2 Ab ELISA

ELISA for Total Antibody to SARS-CoV-2

REF WS-1096 | V. 2020-02US [Eng.] | Σ 96 | IVD

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of WANTAI SARS-CoV-2 Ab ELISA achieved.

For prescription use only.

For in vitro diagnostic use only.

For use under Food and Drug Administration's Emergency Use Authorization (EUA) only.

INTENDED USE

The WANTAI SARS-CoV-2 Ab ELISA is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative detection of total antibodies (including IgG and IgM) to SARS-CoV-2 in human serum and Acid Citrate Dextrose (ACD) plasma. The WANTAI SARS-CoV-2 Ab ELISA is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The WANTAI SARS-CoV-2 Ab ELISA should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform high complexity tests.

Results are for the detection of SARS CoV-2 antibodies. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time 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 positive results to the appropriate public health authorities.

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 for the WANTAI SARS-CoV-2 Ab ELISA may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The WANTAI SARS-CoV-2 Ab ELISA is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY

Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by infection with the SARS-CoV-2 virus. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In severe cases, infection can cause pneumonia, acute respiratory distress syndrome (ARDS), kidney failure and death.

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The 2019 Novel Coronavirus, formerly known as 2019-nCoV and now known as SARS-COV-2, is a new strain of coronavirus that was first identified during 2019-2020 pandemic.

PRINCIPLE OF THE TEST

WANTAI SARS-CoV-2 Ab ELISA is a two step incubation antigen "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant SARS-CoV-2 antigen. The antigen used in the assay is the receptor-binding domain of SARS-CoV-2 spike protein. Patient's serum or plasma specimen is added, and during the first incubation, the specific SARS-CoV-2 antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. Second recombinant SARS-CoV-2 antigen conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) is added, and during the second incubation, the conjugated antigen will bind to the captured antibody inside the wells. The microwells are then washed to remove unbound conjugate, and Chromogen solutions are added into the wells. In wells containing the antigen-antibody-antigen (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after the reaction is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured inside the wells, and to the specimen respectively. Wells containing specimens negative for SARS-CoV-2 antibodies remain colorless.

COMPONENTS

IVD In Vitro Diagnostic Use Only

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

UUU | PLATE **Code 5 (1x96wells)** **8×12/12×8-well per plate**	**MICROWELL PLATE:** Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant SARS-CoV-2 antigen. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C.
CALIBRATOR | - **Code 8 (1x0.5ml per vial)** ***preserv.0.1% ProClin™ 300***	**NEGATIVE CALIBRATOR:** Yellowish liquid filled in a vial with green screw cap. Buffer containing newborn calf serum, tested non-reactive for SARS-CoV-2 antibodies. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
CALIBRATOR | + **Code 7 (1x0.5ml per vial)** ***preserv.0.1% ProClin™ 300***	**POSITIVE CALIBRATOR:** Red-colored liquid filled in a vial with red screw cap. SARS-CoV-2 positive material diluted in buffer containing newborn calf serum. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
HRP | CON **Code 6 (1x12ml per vial)** ***preserv.0.1% ProClin™ 300***	**HRP-CONJUGATE:** Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated recombinant SARS-CoV-2 antigen. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
WASH | BUF | 20X **Code 1 (1x50ml per bottle)** **DILUTE BEFORE USE!** ***detergent Tween-20***	**WASH BUFFER:** Colorless liquid filled in a white bottle with white screw cap. Buffer solution containing surfactant. Once opened, stable for 4 weeks at 2-8°C. The concentrate must be diluted **1 to 20** with distilled/ deionized water before use. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.
CHROM | SOL | A **Code 2 (1x6ml per vial)**	**CHROMOGEN SOLUTION A:** Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
CHROM | SOL | B **Code 3 (1x6ml per vial)**	**CHROMOGEN SOLUTION B:** Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethyl benzidine), N,N-dimethylformamide. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
STOP | SOL **Code 4 (1x6ml per vial)**	**STOP SOLUTION:** Colorless liquid in a white vial with yellow screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

- **PLASTIC SEALABLE BAG:** For enclosing the strips not in use — 1 unit
- **PACKAGE INSERT** — 1 copy
- **CARDBOARD PLATE COVER** — 2 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/600~650nm, microwell aspiration/wash system.

Wantai External Controls (provided separately):

CTRL | NEG **Code N**	**NEGATIVE CONTROL:** Lyophilized in ampoule. Lyophilized, newborn calf serum buffer.
CTRL | POS **Code P**	**POSITIVE CONTROL:** Lyophilized in ampoule. Lyophilized, monoclonal mouse anti-S-RBD antibodies in newborn calf serum buffer.

Preparation of controls: the controls should be reconstituted with distilled or deionized water to the working concentration. Add water into the ampoule according to the volume indicated on the label of the ampoule.

The reconstituted controls can be kept at 2-8°C for no longer than 7 days. For long-term storage, the reconstituted controls should be stored at below -15°C, the freeze-thaw cycles should be not more than 5 times. Before use, balance the reconstituted controls to room temperature and mix well.

Use of controls: add **50µl** of the reconstituted control to the well. One negative and one positive control should be tested for every new lot, or more frequently consistent with the good laboratory practice.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Specimen Collection**: No special patient preparation required. Collect the specimen in accordance with normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into ACD can be tested, but ***highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Specimens with visible microbial contamination should never be used.
3. WANTAI SARS-CoV-2 Ab ELISA is intended ONLY for testing of individual serum or plasma specimens. Do not use the assay for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Specimens can be stored at 2-8°C for one week. For longer-term storage, specimens should be stored frozen (-20°C or lower) for no longer than four weeks with no more than three freeze-thaw cycles. For shipment, specimens should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical specimens and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of WANTAI SARS-CoV-2 Ab ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

The test is time and temperature sensitive. To avoid incorrect results, ***strictly follow the test procedure steps and do not modify them***.

1. This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform high complexity tests.
2. This test has been authorized only for the presence of total antibodies against SARS-CoV-2, not for any other viruses or pathogens
3. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests 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. § 360bbb3(b)(1), unless the authorization is terminated or revoked sooner.
4. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
5. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
6. Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.
7. **CAUTION - CRITICAL STEP**: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
8. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
9. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
10. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
11. After washing of the plate, proceed to the next assay step immediately. Assure same working conditions for all wells.
12. Calibrate the pipette frequently to ensure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contamination.
13. Ensure that the incubation temperature is 37°C inside the incubator.
14. When adding specimens, do not touch the well's bottom with the pipette tip.
15. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600~650nm.
16. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
17. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
18. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Calibrator of the kit. These materials have been tested with tests kits with accepted performance and found negative for HBsAg and antibodies to HIV 1/2, HCV, TP. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative calibrators. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
19. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
20. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
21. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
22. Some reagents like the Stop solution, the Chromogens, and the Wash buffer may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided.
23. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if it comes into contact with the skin or eyes.
24. ProClin™ 300 0.1% used as preservative, can cause an allergic skin reaction. Wipe up spills immediately or wash with water if it comes into contact with the skin or eyes.

INDICATIONS OF DETERIORATION OF THE REAGENT: Values of the Positive or Negative calibrators, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the specimens must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact Wantai technical support for further assistance.

Warning:
H317, H412, P273, P280, P333+P313, P363
ProClin™ 300

Danger:
H360D, P201, P280, P308+P313
N,N-dimethylformamide

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature **(18-30°C)**. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**.

Step 1 **Preparation:** Mark three wells as Negative calibrator **(e.g. B1, C1, D1)**, two wells as Positive calibrator **(e.g. E1, F1)** and one Blank (**e.g. A1**, neither specimens nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step 2 **Adding calibrators and specimen:** Add **50µl** of Positive calibrator, Negative calibrator, and **100µl**

of Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen, Negative Calibrator, Positive Calibrator to avoid cross-contamination.** Mix by tapping the plate gently.

Step 3 **Incubating:** Cover the plate with the plate cover and incubate **at 37°C for 30 minutes**.

Step 4 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step 5 **Adding HRP-Conjugate:** Add **100µl** of HRP-Conjugate into each well except the Blank.

Step 6 **Incubating:** Cover the plate with the plate cover and incubate **at 37°C for 30 minutes**.

Step 7 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step 8 **Coloring:** Add **50µl** of Chromogen Solution A and then **50µl** of Chromogen Solution B into each well including the Blank, mix gently. Incubate the plate **at 37°C for 15 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive calibrator and SARS-CoV-2 antibody positive specimen wells.

Step 9 **Stopping Reaction:** Using a multichannel pipette or manually, add **50µl** of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive calibrator and SARS-CoV-2 antibody positive specimen wells.

Step 10 **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **600~650nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer maintained at the best level of washing performances. In general, no less than **5 automatic washing cycles** of **350-400µl/well** are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out **5 washing cycles**, dispensing **350-400µl/well** and aspirating the liquid for **5 times**. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are disposed of in an appropriate way.
7. The concentrated Wash buffer should be diluted **1:20** before use. If less than a whole plate is used, prepare the proportional volume of solution.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and calibrators. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and calibrators.

Calculation of the Cut-off value (C.O.) = Nc + 0.16
(**Nc** = the mean absorbance value for three negative calibrators). If Nc is < 0.03, take it as 0.03.

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. Negative and Positive Calibrators need to be included in every plate, and their A/CO values must fall within expected ranges for the test results to be valid.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450nm.
- The A values of the Positive calibrator must be ≥ 0.190 at 450/600~650nm or at 450nm after blanking.
- The A values of the Negative calibrator must be ≤ 0.100 at 450/600~650nm or at 450nm after blanking.

If one of the Negative calibrator A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative calibrator A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control
Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative calibrator A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	
Positive calibrator A values after blanking:	1.056	1.082	

All calibrator values are within the stated quality control range

2. Calculation of Nc: = $\frac{(0.020+0.012+0.016)}{3}$ = 0.016. Nc is < 0.03 so the value of 0.03 is used in the next step.

3. Calculation of the Cut-off: (C.O.) = 0.03 + 0.16 = 0.190

INTERPRETATIONS OF THE RESULTS

Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no SARS-CoV-2 antibodies have been detected with WANTAI SARS-CoV-2 Ab ELISA.

Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered positive, which indicates that SARS-CoV-2 antibodies have been detected using WANTAI SARS-CoV-2 Ab ELISA.

WANTAI SARS-CoV-2 Ab ELISA assay result (A/CO)	Result	Test Result Interpretation
<1.0	Negative	Anti-SARS-CoV-2 are not detected
≥1.0	Positive	Anti-SARS-CoV-2 are detected

Confirmation and supplementary testing of any positive specimen with another serology assay is required. The result obtained with the WANTAI SARS-CoV-2 Ab ELISA should not be used to diagnose or exclude acute SARS-CoV-2 infection. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings. A molecular test should be performed if acute COVID-19 is suspected.

PERFORMANCE CHARACTERISTICS

1. Clinical validation study of WANTAI SARS-CoV-2 Ab ELISA was conducted at three sites in China in 2020. 235 subjects confirmed SARS-CoV-2 positive by PCR and 501 subjects confirmed SARS-CoV-2 negative by PCR were tested. All patients who were confirmed positive by PCR exhibited clinical symptoms, laboratory abnormalities or pulmonary imaging manifestations.

WANTAI SARS-CoV-2 Ab ELISA evaluation centers

Clinical institution	PCR Positive	PCR Negative	Total
Site 1	81	273	354
Site 2	39	179	218
Site 3	115	49	164
Total	235	501	736

It was observed that the detection rate of the test was closely related to the time of disease onset, the test showed higher positive detection rate in specimens from patients with long time post onset. Therefore, the interpretation of the test results should consider the specimen's collection time. The WANTAI SARS-CoV-2 Ab ELISA was evaluated with 553 serum samples collected over the course of time from 235 PCR-positive subjects for whom time from symptom onset was known. For performance calculation, when only the results of the first bleed from each patient were considered, the performance of the test was as follows:

Positive percent agreement (PPA) according to days from onset of symptoms based on the first serum specimen collected from each patient

Days from onset of symptoms	Total PCR positive specimens	Number Wantai reactive result	PPA	95% CI
≤ 7	65	36	55.38%	43.34% - 66.83%
8 – 14	92	78	84.78%	76.06% - 90.71%
≥ 15	78	77	98.72%	93.09% - 99.77%

Study design and results of serial bleeds by days from onset of symptoms

Days from onset of symptoms	1st serial results		2nd serial results		3rd serial results		4th serial results		5th serial results	
	No. tests	No. +	No. tests	No. +	No. tests	No. +	No. tests	No. +	No. tests	No. +
0 – 7	65	36	15	11	3	3				
8 – 14	92	78	74	68	36	35	8	8		
≥ 15	78	77	47	46	61	60	50	49	24	24
Total	235		136		100		58		24	

Among 501 PCR-negative subjects, 494 were negative when tested with the WANTAI SARS-CoV-2 Ab ELISA, and the overall Negative Percent Agreement (NPA) was therefore 98.60% (494/501).

2. Independent Clinical Agreement Validation Study: The WANTAI SARS-CoV-2 Ab ELISA was tested on July 2, 2020 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples were confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the WANTAI SARS-CoV-2 Ab ELISA. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers.

All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+". Testing was performed by one operator using one lot of the WANTAI SARS-CoV-2 Ab ELISA. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). Study results and summary statistics are presented in the following tables:

Summary results of the independent evaluation

WANTAI SARS-CoV-2 Ab ELISA	Comparator Method			Total
	Positive (IgM/IgG) +	Negative (IgM/IgG) -	Negative, HIV+	
Positive	29	2	0	31
Equivocal*	1			1
Negative	0	68	10	78
Total	30	70	10	110

Summary statistics of the independent evaluation

Measure	Estimate	Confidence Interval
Sensitivity	96.7% (29/30)	(83.3% - 99.4%)
Specificity	97.5% (78/80)	(91.3% - 99.3%)
Combined PPV for prevalence = 5.0%	67.1%	(33.6% - 88.4%)
Combined NPV for prevalence = 5.0%	99.8%	(99.0% - 100.0%)
Cross-reactivity with HIV+	0.00% (0/10), not detected	

*Note: the package insert used for result interpretation at NCI inadvertently contained an incorrect result interpretation table, which resulted in one sample being classified as Equivocal.

Limitations of the study:

- Samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the real-world performance of the device.
- These results are based on serum and plasma samples only and may not be indicative of performance with other sample types, such as whole blood, including finger stick blood.
- The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.

3. A study was conducted to evaluate the reactivity of the test with potentially cross-reactive specimens containing antibodies against the following viruses and autoimmune conditions.

Specimen	No.	Lot #1		Lot #2		Lot #3		Specificity
		+	-	+	-	+	-	
Flu A	8	0	8	0	8	0	8	100%
Flu B	6	0	6	0	6	0	6	100%
HCV	6	0	6	0	6	0	6	100%
HBV	6	0	6	0	6	0	6	100%
ANA	5	0	5	0	5	0	5	100%
RSV	13	0	13	0	13	0	13	100%
Rhinovirus	6	0	6	0	6	0	6	100%

Specimen	No.	+	-	Specificity
alpha COV 229E	5	0	5	100%
alpha COV NL63	5	0	5	100%
beta COV OC43	7	0	7	100%
beta COV HKU1	4	0	4	100%

LIMITATIONS

1. Use of the WANTAI SARS-CoV-2 Ab ELISA is limited to laboratory personnel who have been trained. Not for point-of care or at-home use.
2. The test is limited to the qualitative detection of anti-COVID-19 antibody in human serum and ADC plasma samples and does not indicate the quantity of antibodies.
3. Performance has only been established with the specimens listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
4. Reactive results must be confirmed with another available method and interpreted in conjunction with the patient's clinical information.
5. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection or to inform infection status.
6. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
7. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history, local disease prevalence, and results of a second but different serology test to confirm an adaptive immune response. Positive results may be due to past or present infection with non- SARS-CoV-2 coronavirus strains such as coronavirus HKU1, NL63, OC43, or 229E
8. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. False positive may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
9. This test is only used for the detection of antibodies to SARS-CoV-2 in human serum and plasma. This test should not be used for screening of donated blood.
10. This test has not been validated for cross-reactivity with anti-*Haemophilus influenzae* positive samples.
11. Antibodies may be below detectable levels in patients who have been exhibiting symptoms for less than 15 days and in some immunosuppressed individuals. Therefore, negative results obtained with WANTAI SARS-CoV-2 Ab ELISA are only an indication that the specimen does not contain detectable level of antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with the virus.
12. False positive results can occur for several reasons related to inadequate washing step. For more information regarding Wantai ELISA Troubleshooting, please refer to Wantai's "ELISAs and Troubleshooting Guide", or contact Wantai technical support for further assistance.
13. The most common assay mistakes include using kits beyond the expiry date, inadequate washing procedures, use of contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation of laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
14. This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.

CONDITIONS OF AUTHORIZATION FOR LABORATORIES

The WANTAI SARS-CoV-2 Ab ELISA Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, 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 WANTAI SARS-CoV-2 Ab ELISA ("your product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

1. 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.
2. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
3. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
4. 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.
5. Authorized laboratories will collect information on the performance of your product and report to Division of Microbiology Devices (DMD)/Office of Health Technology 7 (OHT7) - Office of In Vitro Diagnostics and Radiological Health (OIR)/Office of Product Evaluation and Quality (OPEQ)/Center for Devices and Radiological Health (CDRH) (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Beijing Wantai Biological Pharmacy Enterprise Co., Ltd (wtexport@ystwt.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit and use this 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.
7. WANTAI SARS-CoV-2 Ab ELISA, 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, that meet requirements to perform high complexity tests" as "authorized laboratories."

REFERENCES

1. Ria Lassaunière, et al. Evaluation of nine commercial SARS-CoV-2 immunoassays. doi: https://doi.org/10.1101/2020.04.09.20056325
2. Juanjuan Zhao, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. doi: https://doi.org/10.1101/2020.03.02.20030189
3. Bin Lou, et al. Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. doi: https://doi.org/10.1101/2020.03.23.20041707
4. Fan Wu, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. doi: https://doi.org/10.1101/2020.03.30.20047365
5. Ying Liu, et al. Diagnostic Indexes of a Rapid IgG/IgM Combined Antibody Test for SARS-CoV-2. doi: https://doi.org/10.1101/2020.03.26.20044883

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot- interchangeable.

1. Microwell plate	Code 5	one
2. Negative Calibrator	Code 8	1x0.5ml
3. Positive Calibrator	Code 7	1x0.5ml
4. HRP-Conjugate	Code 6	1x12ml
5. Wash Buffer	Code 1	1x50ml
6. Chromogen Solution A	Code 2	1x6ml
7. Chromogen Solution B	Code 3	1x6ml
8. Stop Solution	Code 4	1x6ml

SUMMARY OF THE ASSAY PROCEDURE:

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add calibrators	50µl
Add specimen	100µl
Incubate	30 minutes
Wash	5 times
Add HPR-Conjugate	100µl
Incubate	30 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	15 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/600~650nm

EXAMPLE SCHEME OF CALIBRATORS / SPECIMENS DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	…										
C	Neg.	…										
D	Neg.											
E	Pos.											
F	Pos.											
G	S1											
H	S2											

MARKING SYMBOLS:

IVD **In Vitro Diagnostic Medical Device**

+2°C~+8°C Storage Conditions

Use By

Content Sufficient For <n> Tests

REF **Catalog Number**

LOT **Batch**

Instructions For Use

Manufacturer

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