COVID19N-REAAD™ Anti-SARS-CoV-2 Nucleocapsid Protein IgG ELISA

CATALOG NO.: CE0100NP1
FOR *IN-VITRO* DIAGNOSTIC USE CE

INTENDED USE

COVID19N-REAAD™ Anti-SARS-CoV-2 Nucleocapsid Protein IgG ELISA (**Coronavirus Disease 2019**–**RE**combinant **A**ntigen-**A**ntibody **D**etection) is an enzyme–linked immunosorbent assay intended for qualitative detection of SARS-CoV-2 IgG antibodies in human serum and plasma (K_2EDTA). COVID19N-REAAD™ is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. COVID19N-REAAD™ should not be used as the sole basis for diagnosis.

COVID19N-REAAD™ is intended for professional use in detecting COVID-19 patients by detecting patient's anti-SARS-CoV-2 antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

The sensitivity of COVID19N-REAAD™ early after infection is unknown. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary. False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

SUMMARY AND EXPLANATION OF TEST

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. The disease was first identified in December 2019, and has since spread globally, resulting in the ongoing pandemic[2,3]. Majority of the cases result in mild symptoms, however some progress to pneumonia and multi-organ failure[2,4]. The overall mortality rate for this disease was up to 4.6% (ranging from 0.2% to 15% according to age group and other health problems)[5].

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons. The incubation time of SARS-CoV-2 is three to seven, maximally 14 days[6].

It has been reported that PCR-confirmed SARS-CoV-2 positive patients may seroconvert and develop antibodies against SARS-CoV-2 antigens anywhere from 6-21days after the onset of clinical symptoms[7]. The specific and reliable detection of human IgG antibodies to SARS-CoV-2 remains a key method to monitor infections, to effect contact tracing, and for serosurveillance.

COVID19N-REAAD™ detects IgG antibodies against SARS-CoV-2 nucleocapsid protein. In conjunction with other diagnostic tests, it can be used to determine if an individual has been exposed to SARS-CoV-2.

PRINCIPLE OF THE PROCEDURE

Recombinant SARS-CoV-2 nucleocapsid proteins are bound onto the microwells. Diluted serum specimens are added to antigen-coated wells and incubated. After incubation and washing, human antibodies
targeting SARS-CoV-2 antigens remain bound to the well surface. Secondary antibody conjugated to horseradish peroxidase (HRP) targeting human IgG is then added to each well. After incubation, the ELISA wells are washed once again before a tetramethylbenzidine (TMB) substrate is added. As the assay determination is based on enzymatic reaction, the reaction can be stopped by the addition of 1N H_2SO_4, which will turn the blue coloration to yellow. The wells can then be read on any suitable spectrophotometer or microwell ELISA plate reader. It is always recommended to read the wells at 450 nm against a 620-630 nm reference filter to eliminate any possible causes of interference.

MATERIALS PROVIDED

The amount of reagents is sufficient for at least 5 optimal runs.

Label	Reagent Constituents	Quantity
MICROPLATE	(Ready to use) 12 strips x 8 microwells coated with SARS-CoV-2 Nucleocapsid protein	1 microplate
20X WASH BUFFER	(20X Concentrate) Phosphate Buffer with 1% Tween 20	50 mL
DILUENT	(Ready-to-use) Phosphate buffer with 1% Bovine serum albumin, 0.05% Tween 20 and 0.05% Proclin 300	2x 50 mL
100X HRP CONJUGATE	(100X Concentrate) Anti-IgG coupled with horseradish peroxidase in stabilizing buffer.	200 µL
TMB SUBSTRATE SOLUTION	(Ready-to-use) Solution with 3,3',5,5' tetramethylbenzidine	15 mL
POSITIVE CONTROL	(100X Concentrate) High level of human chimeric antibody. Preservative: 0.05% Proclin 300	100 µL
NEGATIVE CONTROL	(100X Concentrate) Low level of human chimeric Antibody. Preservative: 0.05% Proclin 300	100 µL
REFERENCE CONTROL	(100X Concentrate) Cut-off level of human chimeric antibody. Preservative: 0.05% Proclin 300	100 µL
STOP SOLUTION	(Ready-to-use) 1N H_2SO_4 solution	15 mL
-	Reseal-able bag for unused microwells	1 piece
-	Instructions for Use	1 copy

PRECAUTIONS

1. For in vitro diagnostic use only.
2. The test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
3. Some countries may regulate this test to be handled at Biosafety Level 2.
4. All components in the test kit have been quality controlled and tested against a Master Lot Unit. Pooling of any component is strictly not recommended; if there is sufficient balance to carry on testing, they should be use wholly on its own and never be pooled.
5. Reagents are only stable up till date of expiry and the manufacturer is not responsible for usage of expired reagents.
6. Do not use the kit if the packaging of components is damaged.
7. Do not use microwell plates if there is no desiccant inside microplate pouch.
8. Assay set up must be carried out at room temperature. Any balance reagents that have been poured out should not be replaced into their original containers in case of cross contamination. All unused portions should be discarded appropriately.
9. The test must be performed on human serum and plasma (EDTA) only. The use of whole blood, or plasma with other anti-coagulants specimen matrices has not been validated.
10. Before opening the Control & Conjugate vials, tap the vials firmly to ensure that the liquids are at the bottom (of the vials).
11. Do not use tap water. Strictly only deionised water can be use whenever required.
12. After each wash, ensure that reagents are added immediately to avoid wells drying up.
13. To avoid cross contamination of reagents, recap containers immediately and change gloves if there are any spills. This is one major reason for false results.
14. Do not mix reagents from different lots within a test run.
15. Do not mix various lots of any kit component within an individual assay.
16. Sodium Azide has been known to inhibit conjugate activity and thus any material that contains the chemical must not be present in the testing area.
17. Over or under washing can result in assay variation and will affect validity of results.
18. Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

ADDITIONAL MATERIALS REQUIRED

1. Measuring containers for wash buffer and diluents.
2. Timer (up to 30 minutes)
3. Calibrated pipettes capable of dispensing 10-200 µL and 200-1000uL with less than 3%CV.
4. Deionised or distilled water.
5. Paper towels.
6. Wash bottle, semi-automated or automated wash equipment.
7. Microplate spectrophotometer with dual wave length. Actual reading at 450 nm with reference of 620~630 nm.
8. Dilution tubes
9. 37°C Dry-Heat incubator

STORAGE AND STABILITY

1. All reagents must be stored at 2 ~ 8°C. Refer to the package for kit expiry.
2. Unopened microwells must be sealed in the reseal-able bag provided and stored at 2 ~ 8°C.

[NOTE: Only with proper and constant storage will kit be stable for the dating period specified.]

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum or plasma (EDTA).
2. Handle all blood samples as if of infectious nature.
3. Restalyst only warrants optimal performance if samples are freshly collected that are clear, non-haemolysed, non-lipemic and non-icteric.

PROCEDURE OF TEST

1. Place the desired number of strips into the microwell frame. Recommended to provide: one well each for Diluent blank, Negative control and Positive control; two wells for Reference control. *If using software for automated system, please check for configuration setup or contact software manufacturer for alternative recommended configuration. Additionally, ensure system is adequately maintained and calibrated appropriately.*
2. Dilute REFERENCE CONTROL, POSITIVE CONTROL, NEGATIVE CONTROL and samples at 100-fold dilution. (2 µL of each to 198 µL of DILUENT).
3. Pipette 100 µL of the respective into the designated well.
4. Incubate the microplate at 37°C for 30 minutes.
5. Meanwhile, prepare diluted HRP Conjugate by diluting 1 volume of 100X HRP CONJUGATE with 99 volumes of DILUENT. (2 µL of HRP to 198 µL of DILUENT).
   Recommended to prepare diluted HRP Conjugate minimally 10 to 20 minutes before adding into well. Diluted HRP Conjugate can be prepared 2 hours before adding into well. For automated system, prepare diluted HRP Conjugate before the start of the test.
   [CAUTION: too early or too late a preparation may yield unstable results]
6. When incubation time is up, wash the wells thoroughly either manually or by using a semi/fully automated washer. Use 300~350µL of 1X Wash Buffer per well. A minimum of 3 wash cycles for manual wash is required while 5 cycles are required for semi/fully automated washers.

Manual Washing:

Fill all wells by dispensing 350µL of 1X Wash Buffer using multichannel pipette. Remove the 1X Wash buffer by flicking out or aspiration. Repeat this step for another 2 times

Automated Washer Settings:

Number of cycles: 3

Dispense Volume: 350µL

Dispense Flowrate: 450µL / Well / Sec

[NOTE: Washing Buffer is prepared by diluting 50mL 20X WASH BUFFER with 950mL distilled water]

7. Ensure that plate is tapped dry; add 100µL of Diluted HRP Conjugate to each well.
8. Repeat steps 4 and 6.
9. When incubation time is up, add 100µL of ready-to-use TMB SUBSTRATE SOLUTION to each well and incubate at 37°C in the dark for 15 minutes.
10. Then add 100µL of STOP SOLUTION to each well and mix well by tapping gently on the sides.
11. Put the microplate in the OD reader to determine the optical density of each well, with primary filter at 450 nm and reference filter at 620~630 nm.

QUALITY CONTROL

For the assay to be valid:

1. The reagent Blank control absorbance should be ⩽ 0.200.
2. The Negative Control absorbance should be ⩽ 0.300.
3. The Reference Control absorbance should be between 0.500 and 0.800.
4. The Positive Control absorbance should be ⩾ 1.200.

CALCULATIONS

Index could be calculated as follow:

$$\frac{OD_{sample} - OD_{blank}}{OD_{Reference} - OD_{blank}} X1 = Index$$

INTERPRETATION OF RESULTS

Calculate the Index value for each test sample as described above (section "CALCULATIONS")

Results are interpreted as follows:

Index < 1: Negative

Index ⩾ 0.9 to <1.1: Inconclusive

Index > 1: Positive

In case of an inconclusive result, a secure evaluation is not possible. It is recommended the samples should be repeated and tested in duplicates. If one or more results are positive, the final interpretation of the specimen is positive; if the repeated results are inconclusive or negative, the final interpretation is inconclusive and another specimen should be collected.

PERFORMANCE EVALUATION

1. Precision

The precision of the Restalyst COVID19N-REAAD™ Anti-SARS-CoV-2 Nucleocapsid Protein IgG ELISA was evaluated with reference to CLSI EP05-A3: Single-Site Prevision Evaluation Study.

Precision

Sample	Mean	Repeatability SD	Repeatability CV	Within Laboratory SD	Within Laboratory CV
Negative Sample	0.24686	0.01246	5.0%	0.01689	6.8%
Reactive Sample	3.61288	0.12210	3.4%	0.15692	4.3%

2. Analytical Specificity

Interfering Substances

The following potential interfering substances were spiked using different concentration into reactive or negative serums against SARS-CoV-2 IgG antibodies, and tested in replicates. No false positivity or false negativity was found in the table below:

Substance	Concentration
Haemoglobin	1.310 g/dL
Total Bilirubin	4.42 mg/dL
Triglycerides	369 mg/dL
Cholesterol	289 mg/dL
Glucose	296 mg/dL
Uric Acid	13 mg/dL
Total Protein	12.1 g/dL
Creatine Kinase	332.3 U/L

Potential Cross-Reacting Analytes

A total of 80 samples containing potentially cross-reacting analytes were tested. Results are shown in the following table:

Indication	N	Reactive
MERS CoV	10	0
SARS-CoV	10	0
Coronavirus NL63	10	0
Coronavirus 229E	10	0
Influenza A	10	0
Influenza B	10	0
RSV (Respiratory Syncytial Virus	10	0
Adenovirus	10	1

3. Clinical Performance

A total of 387 samples were tested which included plasma (K2EDTA) and serum. A total of 56 samples that was derived individual that are positively diagnosed with RT-PCR. Most positive samples (53 out of 56) are collected more than 14 days after diagnosis using RT-PCR.

Methods			RT-PCR		Total
			Pos	Neg	
COVID19N-REAAD™ Anti-SARS-CoV-2 Nucleocapsid Protein IgG ELISA	Reactive	IgG+	55	0	55
	Non-reactive	IgG-	1	331	332
Total			56	331	

This comparison gave the following results:
Positive Percent Agreement (PPA) = 98.21% (55/56) (95%CI: 99.8%-100%)

Negative Percent Agreement (NPA) = 100.00% (331/331) (95%CI: 99.8%-100%)

Overall Rates of Agreement (ORA) = 99.74% (55+331)/ (55+1+331)

LIMITATIONS

1. Optimal assay performance requires the strict adherence to the assay procedure described. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
2. Results obtained from immuno-compromised individuals should be interpreted with caution.
3. The performance characteristics have not been established for visual result determination.
4. The performance characteristics have not been established for matrices other than serum and plasma (K2EDTA).
5. The assay should not be used to diagnose or exclude acute infection. Results are not intended to be used as the sole basis for patient management decisions.
6. 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.
7. A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present during the stage of disease in which a sample is collected.
8. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity.
9. The product has not been tested with samples positive for human coronavirus and other respiratory pathogens antibodies.
10. The assay has not been evaluated with samples collected <14days after symptoms onset.
11. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
12. The sample stability has not been demonstrated.

BIBLIOGRAPHY

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TECHNINCAL PROBLEMS/ COMPLAINTS

Should there be a technical problem/ complaint, please do the following:

1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact Restalyst office or your local distributor.

MEDICAL DEVICE REGISTRATION CERTIFICATE NO./PRODUCT TECHNICAL REQUIREMENT NO.

COVID19N-REAAD™ Anti-SARS-CoV-2 Nucleocapsid Protein IgG ELISA has received Provisional Authorisation from the Health Sciences Authority in Singapore.

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