Evaluation of Encode rapid tests for detection of antibodies against SARS-CoV-2

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CONTENTS:

1 Summary

Background

From the end of 2019, the novel coronavirus disease (COVID-19) outbreak had spread all over China, and is now a global pandemic. Although the virus (SARS-Cov-2) nucleic acid RT-PCR test has become the standard method for diagnosis of SARS-CoV-2 infection, these real-time PCR test kits have many limitations. In addition, high false negative rates were reported. There is an urgent need for an accurate and rapid testing method to quickly identify a large number of infected patients and asymptomatic carriers to prevent virus transmission and assure timely treatment of patients.

Objective

Our aim was to perform an evaluation of the diagnostic performance of Encode rapid tests for detection of antibodies against SARS-CoV-2 and compare their ability to indicate present and past infection in selected clinical settings.

Methods

All participants fulfilled the CDC testing criteria for COVID-19, and samples collected by a swab from the upper airways were tested with PCR against SARS-CoV-2 at a CLIA certified laboratory. We compared and evaluated the antibody detecting ability of the rapid test performance in three arms;

1) 38 institutional patients with either PCR-confirmed positive or negative COVID-19, 16 positive/ 22 negative. Rapid diagnostic tests (RDT) were performed by registered nurses and/or doctors using capillary blood from fingerstick samples.

2) 31 participants sampled by home calls carried out by travel nurses using telemedicine instruction from outside of the room to instruct the patient on self testing. Patients were not currently hospitalized and all were PCR-confirmed COVID-19 positive or negative, 14 positive/17 Negative, RDT tested with capillary blood from fingerstick samples.

3) 5 participants with PCR-confirmed COVID-19 results were tested against ELISA assay over time to determine sensitivity of Encode Lateral Flow Assay against ELISA optical density readings.

Results

All of the institutionalized PCR-confirmed COVID-19 positive patients had detectable IgM and/or IgG antibodies with the Encode RDT tests.. In participants who were contacted via home call with PCR-confirmation of COVID-19, similarly, all patients had detected IgM and/or IgG. In

participants with suspected COVID-19 infection, who were tested simultaneously with PCR and rapid tests, the rapid tests had very high sensitivities and specificities 100% respectively. Elisa Comparisons Show detection ability with the Encode RDT tests above 1.8 OD.

Conclusions and recommendations

In this assessment, Encode rapid tests seem to be very well suited as stand-alone tests to detect present infection in a home health care or institutional care population.

All the tests performed were able to detect either the presence or lack of SARS-CoV-2 antibodies. To confirm past infection beyond 60 days post infection, we recommend the use of ELISA laboratory testing instead of the Encode tests as IgG levels are most often decreased below the threshold for the RDT sensitivity.

2 Background

In December 2019, Wuhan city in Hubei Province, China, became the center of an outbreak of a severe pneumonia, later identified as caused by a novel coronavirus SARS-CoV-2 [1]. Human-to- human transmission of SARS-CoV-2 occurs primarily through respiratory droplets. Due to the rapid spread of the virus WHO declared SARS-CoV-2 a worldwide pandemic by February 2020. As of July 30th, there were 17,295,529 cases and 673,169 deaths confirmed worldwide [2]. The clinical presentation of COVID-19 varies from asymptomatic disease, mild upper respiratory infection to severe pneumonia with respiratory failure and death as well as patients with mild symptoms who later are hospitalized with severe blood clotting.

Laboratory methods for diagnosing COVID-19

Currently in the US, COVID-19 is diagnosed by detection of SARS-CoV-2 RNA with rtPCR in a sample collected by a swab from the upper airways. PCR is performed at medical microbiology laboratories, requiring advanced analytical instruments and trained personnel. Together with growing concerns regarding shortage of sampling equipment and necessary reagents, this limits the number of people currently being tested for COVID-19.

Detecting humoral immune response to the virus is a different analytical approach. Generally, immunoglobulin type M (IgM) is produced during the early stages of an infection, usually followed by production of immunoglobulin type G (IgG). For infection with the SARS-CoV-2 virus, however, there is some evidence that IgG may be detected at the same time as IgM, or

even earlier [3].

Several enzyme immunoassay (EIA) methods to detect and quantify antibodies against SARS-CoV-2, both Laboratory and RDT, are available at hospitals and laboratories. At the same time, there is still a substantial need for more accurate, cost effective and easier to use rapid diagnostic tests. These rapid tests are for professional use, they make use of capillary or venous whole blood, plasma or serum, and are designed to qualitatively detect IgM and/or IgG antibodies against SARS-CoV- 2. The results are read visually after 10-15 minutes. Even though there are other rapid tests in the market, they generally come with very limited documentation on test performance, and with a few exceptions without any manufacturer independent evaluation [4, 5]. To determine a rapid test's ability to detect past infection, its performance with regard to IgG antibodies is emphasized by the United Kingdom Medicines & Healthcare products Regulatory Agency [6].

3 Objectives

Our main objective was to perform a comprehensive evaluation of the diagnostic accuracy of a Encode rapid test for COVID-19. In particular, we aimed to evaluate if these tests could be used as a stand alone method to accurately and effectively diagnose patients. The project was designed as a clinical study to compare the performance of this assay with rtPCR and ELISA in selected clinical settings and clinical instruction via telemedicine.

4 Methods

All rapid tests were performed in accordance with manufactures' instructions [Table 6] under optimal and standardized conditions, with two distinct patterns:

1 by experienced medical clinicians, using capillary blood samples and

2 by clinical instruction via telemedicine using capillary blood samples via finger prick.

Study design We evaluated the performance of the point-of-care rapid tests in three study arms*:*

1. 38 patients of Washington county Correctional Facility and medical staff at washington county correctional facility in Utah, USA, with PCR-confirmed COVID-19.

Positive by rtPCR (16 patients)

Days since onset of symptoms:

- <7 days for two patients
- 7+ days for 14 patients

Negative by rtPCR (22 Patients)

Days since onset of symptoms:

- <7 days for four patients
- 7+ days for 18 patients

2. 35 participants sampled by home calls carried out by travel nurses using telemedicine instruction.

Positive by rtPCR (18 patients)

Days since onset of symptoms:

- <7 days for four patients
- 7+ days for 14 patients

Negative by rtPCR (17 Patients)

Days since onset of symptoms:

- <7 days for two patients
- 7+ days for 15 patients

3. 5 previously rtPCR participants sampled by home calls carried out by travel nurses using telemedicine instruction and Stratified by ELISA.

Positive by ELISA and rtPCR (5 patients)

Days since onset of symptoms:

- <30 days for 5 patients

Test performed as degrading immunoglobulins

RDT/LFA Encode procedure at point of care.

1.

Extract capillary whole blood specimen by firmly pressing lancet against finger.

- Kit performed on whole blood capillary specimen extracted via lancet on medial distal surface of the ring finger.
- Testing is performed immediately after specimen collection.

2.

Whole Blood sample and Buffer Diluent is placed on the Lateral Flow Assay

- Testing Device is placed on a clean flat surface. Fill the pipette dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 10 µL) of whole capillary blood into the sample well, making sure that there are no air bubbles. Then add 1-2 drops (about 70-100 µL) of Sample Diluent immediately.

3.

Timer is started. Results checked at 5 minutes, 10 minutes and final read at 15 minutes.

For arm 3 of testing, participants consented to having capillary whole blood drawn and placed in one tube of K_2-EDTA to be used for evaluation of the ELISA test. At intervals of every 30 days.

Statistical analyses

rtPCR and ELISA results from the CLIA certified laboratories were used as comparison when investigating diagnostic accuracy of the rapid tests. In all arms, we calculated the tests' sensitivities (positivity rates) and specificity (negative rates). Sensitivity was defined as the proportion of patients diagnosed with COVID-19 using rtPCR on respiratory samples, who had detectable IgM or IgG antibodies on the rapid tests. We also calculated specificity, defined as the proportion of participants with negative rtPCR tests who were also antibody negative. Further we stratified positivity rates ELISA optical density. IgM and IgG test results were evaluated separately, which detected "total antibodies". Because sample sizes were relatively small but results were consistent, 90% confidence intervals for binomial proportions were calculated using the adjusted Wald method [7].

Ethical considerations

The project was approved by the data protection and ethics officials at each test arm. Oral and

written consent was obtained from participants in all study arms.

5 Results

Results from institutional participants (arm 1) showed that the 16 tests detected IgM and IgG antibodies in this population which were also confirmed positive by rtPCR, and 22 participants did not have detectable IgG or IgM which were subsequently confirmed negative by rtPCR. (Tables 1 and 2).

Arm 2 consisted of 35 participants sampled by home calls carried out by travel nurses using telemedicine instruction.

Of the 35 participants in arm 2, 18 had positive PCR tests and positive IgG and IgM RDT tests. Also in arm 2, 17 tested PCR negative and also had negative RDT tests. In this population, the rapid tests had very high sensitivities when compared to PCR (Tables 2 and 3). Positivity rates, especially for IgM, increased with increasing number of days since onset of symptoms (Table 4), although numbers were too small to draw firm conclusions. Since no rapid tests were positive in participants with negative PCR tests, calculated sensitivities and specificities were high.

Not all participants with confirmed COVID-19 by rtPCR had detectable antibodies. Also, despite comparable sensitivities, the rapid tests did not necessarily give the same result in all participants. IgM and IgG results were identical in all participants in arms 1, 2 and 3, leaving the question of whether the test distinguishes between IgM and IgG.

One patient was excluded from the study. The patient was self-reported to have positive rtPCR for COVID-19 at 62 days prior to entrance into the study. His ELISA was positive OD at the cutoff of 0.1 for IgG to COVID-19. The Encode RDT did not show positive for IgG or IgM. While this patient was excluded from the study as we could not confirm the positive rt-PCR COVID-19 status, it appears that low levels of IgG if longer than 60 days since infection might be a limiting factor. Although the number for this study in this category is too low to draw firm conclusions.

6 Discussion

PCR for detection of viral RNA and antibody detection tests use different test principles and are

not interchangeable. Early in the infection, we expect PCR to be positive and antibody detection tests to be negative. As the infection progresses and clears, most patients will develop detectable antibodies, while the virus is gradually cleared from the body. Thus, even under the best of circumstances, PCR is far from an ideal “gold standard” for comparison of the rapid tests.

If a participant with PCR-confirmed COVID-19 has no detectable antibodies, there are several possibilities: (i) the stage of the infection is too early for antibodies to have been formed, (ii) the level of antibodies produced is too low to be detected, (iii) the participant does not form antibodies ([3, 8]), (iv) a false negative rapid test result, or v) a false positive PCR result (wrong labeling for instance). Similarly, if a participant with negative SARS-CoV-2 PCR has detectable antibodies on a rapid test, there are a number of plausible explanations: (i) false positive rapid test result (for instance cross reaction with other antibodies), (ii) false negative PCR result (pre-analytical or analytical issues), or iii) the participant recovered from COVID-19 and cleared the virus prior to PCR testing. Comparing results from several rapid tests with each other may provide some clue as to which is the most likely explanation in each case, but until we have a gold standard method for antibody detection, we cannot be certain which is true.

In our study, IgG and IgM positivity rates in arm 1 (institutional patients) and arm 2 (recovered, community treated participants) were identical. This was mostly expected as arm 1 patients were not hospitalized but institutionalized. However, it is an interesting finding that community treated patients had the same detection limits as institutionalized patients. This is in respect to previous studies which have shown that hospitalized patients are likely more severely affected, and more severe infection has been associated with higher levels of antibodies [3, 9]. It is also worth noting that according to manufacturers’ information, most of the rapid tests have been evaluated in samples from hospitalized populations. This would infer that institutionalized populations have similar antibody profiles to community populations.

Most COVID-19 recovered participants should have had sufficient time to develop IgG antibodies, as median seroconversion time has been reported at around 13-14 days after onset of symptoms [3, 9]. The rapid tests displayed high degrees of IgG and IgM positivity rates in this population. One might speculate that antibody levels were high in this group, who all had symptoms and positive rtPCR tests, which may affect rapid test performance. We were not able to evaluate the tests’ performances in a population that had been through a COVID-19 infection with little or no symptoms.

In our study most participants had a short duration of severe symptoms. All of the rapid tests in our study had high sensitivities compared to PCR in this population, confirming expectations. It is possible that the rapid tests, and IgM in particular, may still have a supplemental role in diagnosing COVID-19 in the acute phase. In any case, an isolated positive IgM-only result should be followed by a second sample to detect IgG-seroconversion and thereby rule out the

possibility of an unspecific IgM result. Furthermore, a negative result in the acute phase of infection should never be used to exclude COVID-19.

In arm 3, we also calculated specificities of the rapid tests. We do not know if a participant with a negative PCR test and a positive IgG rapid test is someone who has recovered from COVID-19, or if the rapid test result is a false positive. We therefore have to be cautious when interpreting calculated specificities, and in the present study, we do not evaluate the tests based on this parameter.

7 Conclusions and recommendations

All tests were able to detect SARS-CoV-2 antibodies in participants with PCR-confirmed COVID-19, although positivity rates were generally more clearly defined in the more severely affected patients. As the sample size was adequate for this purpose we feel that we can confidently recommend the Encode platform for use in the fight against the COVID-19 pandemic. We still however acknowledge that further studies may be warranted to assess the usefulness of antibody rapid tests in the acute phase of COVID-19 as a supplement to rtPCR.

When a rapid test is used to confirm past COVID-19 infection, we recommend using Encode as it is a test with high IgG positivity rates in recovered COVID-19 patients, in line or better than the specification criteria for serology point of care tests published by both the US Food and Drug administration as well as the United Kingdom Medicines & Healthcare products Regulatory Agency [6]. In our study, it seems that Encode RDT's (Table 1) fulfill these recommendations, under the assumption that their specificities are high. In a population where the prevalence of COVID-19 is low, however, there is a risk of false positive results, which we cannot at present quantify because of zero false positives and the low numbers in this study. We acknowledge this limitation of the study, in addition to the limited sample size, and our results are therefore preliminary and must be interpreted with caution.

Many rapid tests are now marketed with very limited documentation thus we have undertaken to study the efficacy in the most clinically relevant settings. Our results show high sensitivity and specificity in these settings.

Due to the similar outcomes between institutionalized tests administered by a registered nurse (arm 1) and the telemedicine guided tests (arm 2) we recommend the encode LFA platform for use in rural or native populations where on site testing may be limited.

As a negative antibody test performed during the early phase of infection cannot rule out COVID-19, we recommend not using a rapid test until at least 7 days after exposure or at least 2 days after onset of symptoms. A negative test may be repeated, but not all COVID-19

infected patients develop antibodies, and not all antibodies are necessarily detected by the rapid test. Thus, a negative rapid test does not rule out current nor past COVID-19 disease and follow up testing may be warranted.

We highly recommend performing an independent evaluation before using any test including the various forms of rtPCR equipment as the Covid-19 research is fluid and ongoing. It is important to evaluate the test performance in the population it is intended for. Thus, when a rapid test is to be used to detect past infection in people who have not had symptoms within the last 60 days, it is not sufficient to validate the test in an institutionalized known population. As more information becomes available about the efficacy of rtPCR and ELISA methodologies we will use ELISA as a comparison method in addition to rtPCR. This will allow us to investigate diagnostic accuracy and analytical properties of the rapid tests more thoroughly.

In our study, with a limitation of sample size we were able to detect SARS-CoV-2 antibodies at a sensitivity of 100% (table 2) However, it is unlikely that in a larger sample size this same accuracy would be achieved. Although the true sensitivity and specificity of this test may be greater than 99% it is very difficult to account for a patient with poor innate immune response or any number of compounding physiological anomalies.

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9 Tables

Table 1. Results of Encode rapid flow assay (LFA)

	rtPCR Positive	rtPCR Negative	Total
LFA Positive	34	0	34
LFA Negative	0	39	39
Total	34	39	73

Table 2. Results of Encode rapid flow assay (LFA)

	Outcome	Confidence Interval
Sensitivity	34/34 = 100%	94.5 - 100
Specificity	39/39 = 100%	95.2 - 100

ELISA Sensitivity	>1.8 OD	

10 Appendix

Appendix A. Prospective Instruction from manufacturer.

SARS-CoV-2 IgG/IgM Rapid Test
Package Insert

Cat: RCD-422 **Specimens:** Whole Blood/Serum/Plasma

Version:13 **Effective Date:** 2020-04

Read this package insert completely before using the product. Follow the instructions carefully when performing testing. Not doing so may result in inaccurate test results. Before performing testing, all operators MUST read and become familiar with Universal Precautions for Prevention of Transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease (COVID-19), and other Blood-borne Pathogens in Health-Care Settings.

The SARS-CoV-2 IgG/IgM Rapid Test (Whole Blood/Serum/Plasma) is a lateral flow immunoassay for the qualitative detection and differentiation of IgG and IgM of Novel Coronavirus (SARS-CoV-2) in human whole blood, serum or plasma.
This test is intended to be used as a screening test and as an aid in the diagnosis of infection with Novel Coronavirus. Any reactive specimen with the SARS-CoV-2 IgG/IgM Rapid Test must be confirmed with alternative testing method(s).
Coronavirus (SARS-CoV-2) Belongs to the genus Nestovirus, Coronaviridae, and is divided into four genera: α, β, γ and δ. The α and β gene are only pathogenic to mammals. The γ gene mainly causes bird infections. The δ gene include nightingale coronavirus HPU11, thrush coronavirus HPU12, munia coronavirus HPU13. CoV is mainly transmitted through direct contact with secretions or through aerosols and droplets. There is also evidence that it can be transmitted through the fecal-oral route.
So far, there are 7 types of human coronaviruses (HCoV) that cause human respiratory diseases: HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1, MERS-CoV and Novel Coronavirus (SARS-CoV-2) (2019), it's an important pathogen of human respiratory infections. Among them, a SARS-CoV-2 was discovered in 2019 due to Wuhan virus pneumonia cases. The clinical manifestations are systemic symptoms such as fever and fatigue, accompanied by dry cough, dyspnea and so on. Can quickly develop into severe pneumonia, respiratory failure, acute respiratory distress syndrome, septic shock, multiple organ failure, severe acid-base metabolism disorders, etc., and even life-threatening.
The SARS-CoV-2 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *SARS-CoV-2* antigen conjugated with colloid gold (*SARS-CoV-2* conjugates) and quality control antibody gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (IgG and IgM bands) and a control band (C band). The IgG band is pre-coated with monoclonal anti-human IgG for the detection of IgG anti- *SARS-CoV-2*, IgM band is pre-coated with reagents for the detection of IgM anti- *SARS-CoV-2* and the C band is pre-coated with quality control antibody.
When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. *SARS-CoV-2* IgM antibodies if present in the specimen will bind to the *SARS-CoV-2* conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM antibody, forming a burgundy colored IgM band, indicating *SARS-CoV-2* IgM positive test result.
SARS-CoV-2 IgG antibodies if present in the specimen will bind to the *SARS-CoV-2* conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy colored IgG band, indicating a *SARS-CoV-2* IgG positive test result.
Absence of any test bands (IgG and IgM) suggests a negative result. The test card also contains a quality control band C. Regardless of the presence or absence of a detection band, the red quality control band C should appear. The quality control band is a color band of the quality control antibody immune complex. If the quality control band C does not appear, the test result is invalid, and the sample needs to be tested again with another test card.

Materials Provided

· Test devices · Droppers

· Buffer · Package insert

Materials Required but Not Provided

- Centrifuge (for plasma only)
- Timer
- Specimen collection containers

- For professional *in vitro* diagnostic use only.
- Expiry date is 24 months after its manufacture date . Do not use the test if its foil pouch is damaged. Do not reuse tests.
- The kit should be stored at 2〜30°C in cool and dry place, protected from light.
- After open the aluminum foil pouch, the test card will become invalid due to moisture absorption. Please use it within 1 hour.

- The kit can be performed used on Serum, Plasma or Whole Blood specimen, include plasma or whole blood samples prepared from commonly used anticoagulants (EDTA, heparin, sodium citrate).
- Testing should be performed immediately after specimen collection. If it cannot be detected immediately, the serum and plasma specimen to be tested can be stored at 2 〜 8 ° C for 5 days. For long-term storage, store at -20 ° C. Avoid repeated freeze-thaw specimens. Anticoagulated whole blood specimens should not be stored for more than 72 hours at room temperature; not more than 7 days at 2-8 ° C.
- Before testing, slowly return the refrigerated or frozen specimens to room temperature and mix them carefully. When clearly visible particulate matter is present in the specimen, it should be centrifuged to remove sediment before testing.
- If the specimen contains a large amount of lipid, hemolysis or turbidity, please do not use it, so as not to affect the result judgment.

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Place the test device on a clean, flat surface and label specimen number
2. Fill the pipette dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 10 μL) of whole blood , serum, plasma into the sample well, making sure that there are no air bubbles. Then add 1-2drops (about 70-100 μL) of Sample Diluent immediately.
3. Set up timer. Results can be read in 15 minutes. ***Don't read result after 15 minutes.***

POSITIVE RESULT: In addition to the presence of C band, if only IgG band is developed, the test indicates for the presence of *SARS-CoV-2* IgG antibody. The result is positive.

In addition to the presence of C band, if only IgM band is developed, the test indicates for the presence of *SARS-CoV-2* IgM antibody. The result is positive.

In addition to the presence of C band, both IgG and IgM bands are developed, the test indicates for the presence of both IgG and IgM anti-*SARS-CoV-2,* The result is also positive.

***NOTE:** Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

NEGATIVE RESULT: If only the C band is present, the absence of any burgundy color in the both test bands (IgG and IgM) indicates that no *SARS-CoV-2* antibody is detected in the specimen. The result is negative.

INVALID RESULT:

If no C band is developed, the assay is invalid regardless of any burgundy color in the test bands. Repeat the assay with a new device.

1. Positive Coincidence Rate: The test results of positive quality control are all positive
2. Negative Coincidence Rate: The test results of negative quality control are all negative
3. Analytical Specificity: The test results of specimen from non- infected by novel coronavirus should be negative
4. Analytical Sensitivity: The detection result is positive when detection of a novel coronavirus IgG strongly positive serum 1:50 dilution sensitivity reference : The detection result is positive when detection of a novel coronavirus IgM strongly positive serum 1:50 dilution sensitivity reference
5. Intra-Assay: There is no different test results of the same quality control in the same batch;
6. Inter-Assay: There is no different test results of the same quality control from different batch.

1. The Assay Procedure and the Assay Result Interpretation must be followed strictly the inserts when testing. Failure to follow the procedure may give inaccurate results.
2. This kit is only used for in vitro diagnosis and is only used for qualitative detection of Coronavirus IgG and/or IgM antibodies in blood samples, and cannot be quantitative detection.
3. Positive and negative results indicate the presence of IgG and/or IgM antibodies with/without detectable concentrations of Coronavirus in blood samples, but cannot be used as the sole criterion for the determination of Coronavirus infection. Other methods (such as nucleic acid testing) should be used for identification when necessary, and comprehensive judgment should be made based on the test results.
4.It is only provided for use by clinical laboratories or to healthcare workers for point-of care testing, and not for at home testing. The test package includes instructions for use along the following lines:
5.It is only used as a supplementary test indicator for SARS-CoV -2 nucleic acid negative suspected cases or in conjunction with nucleic acid detection in the diagnosis of suspected cases. Not applicable to screening in the general population.
6.Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
7.Positive results should be confirmed by additional testing and clinical evaluation under the guidance of the physician.

8.It is used to detect SARS-CoV-2 IgM and IgG antibodies in human blood samples. It is possible to get any results from other liquid samples, and the above results cannot be used as a judgment criterion.
9..There are individual differences in the time of antibody production after SARS-CoV-2 infection, so the window period from SARS-CoV-2 infection to antibody detection is not consistent among different individuals.
10.It is not for the screening of donated blood.

1.Before using the kit, please read the instructions carefully and control the reaction time strictly. If you do not follow the instruction, you will get inaccurate results.
2. The specimen should be tested in the laboratory with certain conditions.All samples and materials in the testing process shall be handled in accordance with the laboratory practice for infectious diseases.
3. Be careful to prevent the product from getting wet, and do not open the aluminum bag before it is ready for testing;If the aluminum foil bag is damaged or the test card is damage, it cannot be used.
4. Do not replace the components in this kit with components in other kits.
5. Hemolytic specimen should not be used for testing.
6. Do not use cloudy pollution specimen for testing.
7. Do not dilute the specimen for testing, otherwise inaccurate results may be obtained.
8. The kit shall be stored in strict accordance with the conditions specified in this manual. Please do not store the kit under freezing conditions.
9. This kit is limited to qualitative detection of Coronavirus antibodies in human serum, plasma or whole blood.
10. The kit will produce negative results under the following conditions: when the titer of the Coronavirus antibody in the specimen is less than the minimum detection limit of the kit, or the Coronavirus antibody does not exist at the time of specimen.
11.Specimen contained higher titers of heterophobic antibodies or rheumatoid factors may affect the expected results.
12.When the assay procedure is completed, in accordance with local regulations dispose the test kit and tube

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	Catalog number		Temperature limitation
	Consult instructions for use		Batch code
	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse		Authorized representative in the European Community
	CE marked according to IVD Medical Devices Directive 98/79/EC		

Zhuhai Encode Medical Engineering Co.,Ltd
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Tel: (747) 333-8388
E-Mail: cs@evanla.com
Website: www.evanla.com

Important FDA guidance:

This test has not been reviewed by the FDA.

Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.

Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.

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.

Not for the screening of donated blood

MATERIALS REQUIRED BUT NOT PROVIDED

Timer or watch capable of timing 20
Clean, disposable, absorbent workspace cover
Biohazard waste container
Additional items required for fingerstick and venipuncture whole blood collection, and plasma specimens:
Antiseptic wipe
Sterile lancet to obtain a fingerstick whole blood specimen, or materials required to obtain a venipuncture whole blood specimen
Sterile gauze pads
Latex, vinyl or nitrile disposable gloves
Centrifuge to process a plasma specimen

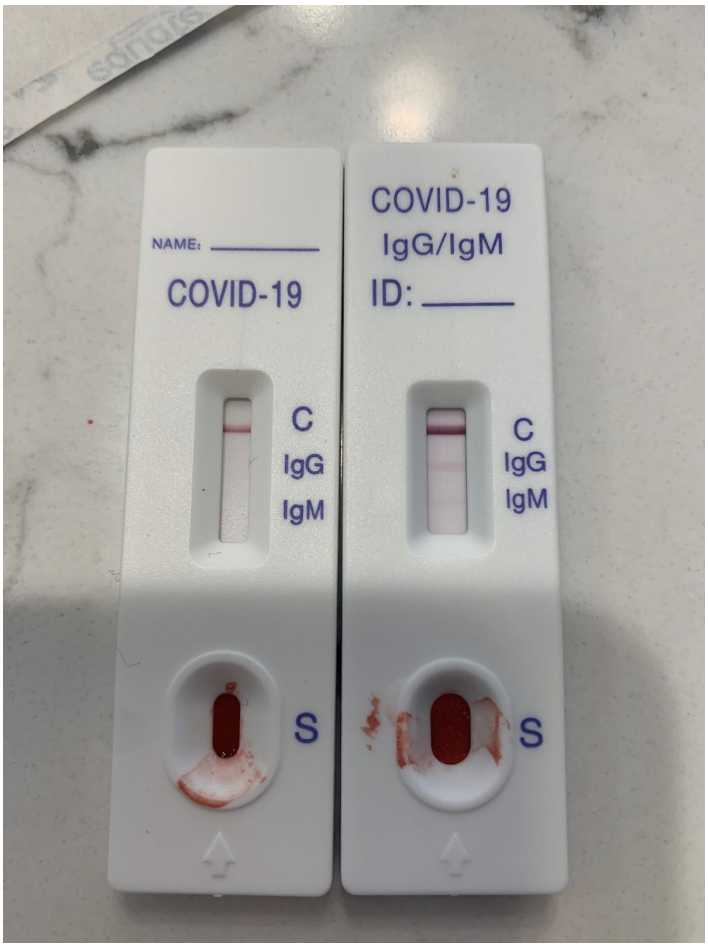
WARNINGS

For *in vitro* Diagnostic Use
1. Read the package insert completely before using the product. Follow the instructions carefully. Not doing so may result in inaccurate test results.
2. Before performing testing, all operators MUST read and become familiar with Universal Precautions for Prevention of Transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease (COVID-19), and other Blood-borne Pathogens in Health-Care Settings. Pathogens in Health-Care Settings.
3. This test should be performed at temperatures in the range of (15°- 37°C, 59°- 99°F). If stored refrigerated, test is brought to operating temperature (15°- 37°C, 59°- 99°F) before performing testing.
4. If the test kit is stored at temperatures outside of ambient temperature (2°- 27°C, 36°- 80°F), or used outside of the operating temperature (15°- 37°C, 59°- 99°F).
5. Individuals undergoing preventive treatment for SARS-CoV-2 may produce false negative results.

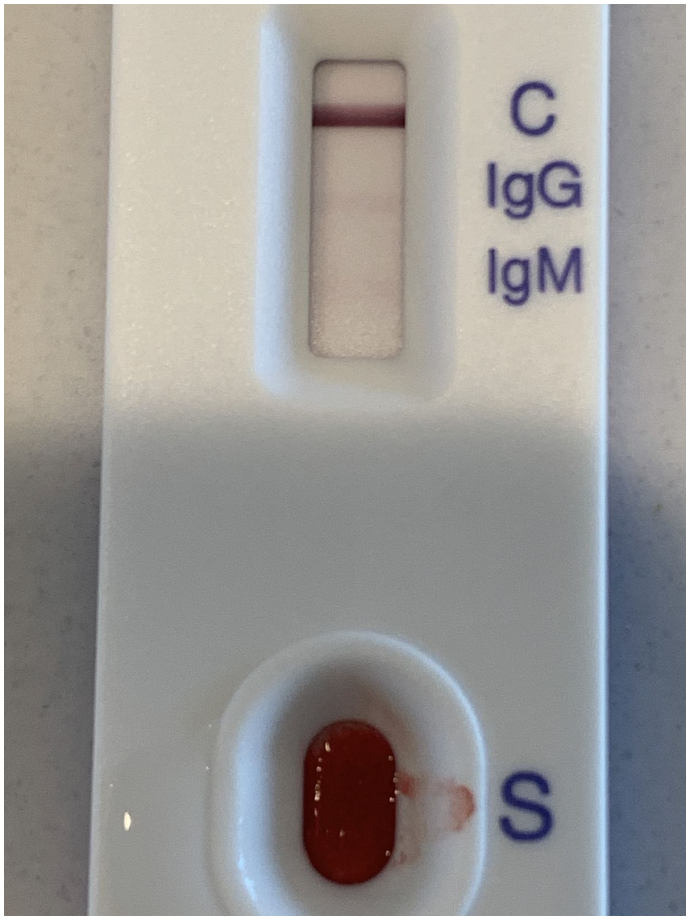
PRECAUTIONS

Safety Precautions
1. Handle blood specimens and materials contacting blood specimens as if capable of transmitting infectious agents.
2. Do not drink, eat, or smoke in areas where specimens are being handled or testing is being performed.
3. Wear disposable gloves while handling blood specimens and performing testing of blood specimens. Change gloves and wash hands thoroughly after performing each test. Dispose of used gloves in a biohazard waste container.
4. Dispose of all test specimens and materials used in the test procedure in a biohazard waste container. Lancets and venipuncture materials should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Liquid wastes may be mixed
with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination.
NOTE: Do not autoclave solutions that contain bleach.
5. Wipe all spills thoroughly with a solution of 10% bleach or other appropriate disinfectant4. Bleach solutions should be made
fresh each day.
Handling Precautions
1. Use all Test Devices only once and dispose of properly (see Safety Precautions). **Do not reuse any of these test components.**
2. Do not use the test beyond the expiration date printed on the box. Always check expiration date prior to testing.
3. Do not interchange Test Devices and Buffer Solutions from kits with different lot numbers.
4. Avoid microbial contamination and exercise care in handling the kit components.
5. Adequate lighting is required to read a test result.

Appendix B. Detection on LFA for Elisa (arm 3) at lowest point of detection



Right is Encode LFA at >1.8, Left is same blood sample with other publicly available LFA kit.



Encode Kit at lowest detection point >1.8 OD