THERATESTLABS
Advanced Immunoassays & More

EL-Anti-SARS-CoV-2

Catalog Nos.:
104-128 (EL-Anti-SARS-CoV-2 IgM/IgG, 2 plate)
104-129 (EL-Anti-SARS-CoV-2 IgM)
104-130 (EL-Anti-SARS-CoV-2 IgG)

Instruction Manual

An enzyme immunoassay for the
qualitative detection with numerical expression of IgM and IgG class antibodies against SARS-CoV-2 in human serum

For professional use only

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INTRODUCTION

Intended use

For in-vitro diagnostic use

The **TheraTest EL-Anti-SARS-CoV-2 IgM/IgG** is an enzyme immunoassay for the qualitative detection with numerical expression of IgM and IgG class antibodies in human serum against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) associated with the coronavirus disease 2019 (COVID-19), a respiratory illness.

Summary and Explanation

COVID-19 is a respiratory illness caused by SARS-CoV-2 virus, a betacoronavirus related to the viruses causing the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003 and Middle East Respiratory Syndrome (MERS) outbreak in 2012[3]. Entry of betacoronaviruses into human cells is facilitated by the spike (S) protein, which is comprised of two subunits, S1 and S2. The S1 subunit contains the receptor binding domain (RBD), which binds to Angiotensin-Converting Enzyme 2 (ACE2) in human lung tissue[2]. Early studies of SARS-CoV-2 serology have shown ELISA testing for antibodies against the S protein RBD and nucleocapsid (N) protein to have higher sensitivity for detecting patients with mild symptoms, compared to the complete S1 subunit[3]. The RBD region of the SARS-CoV-2 S1 protein was selected for use as the antigen in this assay for its reported increased sensitivity to detect antibodies in patients with mild symptoms and for the reported neutralizing capability of RBD-targeted antibodies in patients infected with the related SARS-CoV virus[8].

Longitudinal studies of immunity to SARS-CoV following the 2003 outbreak found that IgM was detectible in human serum 3-5 days after onset of illness with seropositivity peaking at one month post-exposure. Serum anti-SARS IgG levels were detectable in the majority of the population studied at two weeks post exposure, with seropositivity lasting up to three years[7]. Cross-reactivity anti-SARS antibodies with SARS-CoV-2 antigens has been demonstrated in vitro. However, as Wu et. Al observed, the seronegativity of 91% (21/23) of patients exposed to SARS-CoV upon repeat testing at 6 years post-exposure indicates that this cross-reactivity is unlikely to contribute significantly to the rate of false-positives when testing the general population[3][7].

Principle of the procedure

The **TheraTest EL-Anti-SARS-CoV-2 IgM/IgG** assay is a solid phase enzyme immunoassay for the qualitative detection of anti-CoV-2 antibodies. The wells of 96-well polystyrene plates have been coated with recombinant SARS-CoV-2 S protein RBD antigen. The wells are incubated with Calibrators, Controls, and diluted serum specimens. During the incubation, the antibodies present in the test sample bind to the solid phase antigen. Then the wells are then washed and horseradish-peroxidase labeled anti-human IgM ($Fc_5\mu$ specific) and IgG (Fcγ specific) is incubated in the wells. Unbound anti-IgM and anti-IgG antibodies are removed by aspiration and washing. A specific chromogen substrate is added to the wells and the

autoantibodies + anti-IgM/G complex is detected by a resulting color change, which is measured by a spectrophotometric enzyme immunoassay reader. A direct relationship exists between the amount of anti-SARS-CoV-2 antibodies in the specimen and the absorbance value detected by the spectrophotometer[(1)]. Results are reported as Units (qualitative method with numerical expression) based on the value of the Calibrators (IgM and IgG) provided.

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only

Reagents Containing Human Source Material

Calibrators and control materials contain human serum. Treat as potentially infectious. Calibrator and control materials contain heat-inactivated human serum. While these methods are highly accurate, no sterilization method can offer complete assurance that HIV, hepatitis virus or other infectious agents are absent. Therefore these materials and all patient specimens should be handled as though capable of transmitting infectious diseases. Human material should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories 5th Edition", 1 December 2009 (2009-12-01), pages 1 – 416.

Web site: https://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

Stop Reagent (2 mol/L Phosphoric Acid)

Corrosive! May cause severe burns upon contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amount of water for at least 15 minutes.

Hazardous Substance Risk & Safety Phrases:

R34 - Causes burns.
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection
S45 - In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Chromogen

Irritant! This product contains 3,3',5,5'-tetramethylbenzidine (TMB) (≤0.05%), a chromogenic indicator of horseradish peroxidase activity. It has shown neither mutagenic nor carcinogenic effects in laboratory experiments (15).

Hazardous Substance Risk & Safety Phrases:

R36/37/38 – Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.
S24/25 – Avoid contact with skin or eyes.
S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36 – Wear suitable protective clothing.
S51 – Use only in well-ventilated areas.

Reagents Containing Sodium Azide

Calibrators and Controls contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush drain with large quantities of water to prevent azide build-up.

Hazardous Substance Risk & Safety Phrases:

R22 - Harmful if swallowed.
R36/37/38 - Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 - After contact with skin, wash immediately with plenty of water.
S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection
S46 - If swallowed, seek medical advice immediately and show this container label.

General Precautions and Information

1. Do not pipette by mouth.
2. Do not eat, drink, or smoke in designated work areas.
3. Wash hands thoroughly after using specimens and kit reagents.
4. Do not use test components beyond the expiration date.
5. Work in a well-ventilated area when using kit reagents.
6. Avoid exposing reagents to excessive heat or light during storage.
7. Do not allow the Chromogen to come in contact with metal or oxidizing agents.
8. Use disposable glassware and plasticware or wash all material thoroughly according to standard laboratory practice.
9. Calibrators and Controls are lot specific and therefore are not interchangeable among kits of different lot numbers.
10. Avoid microbial contamination of the reagents.
11. Dispose of containers and unused kit reagents in accordance with local regulatory requirements

COVID-19 Disclaimers

1. This test has not yet been reviewed by the FDA. Performance data has been submitted to the FDA's CDRH in accordance with Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) section D, published in May 2020.
2. Negative results do not rule out SARS-CoV-2 infection, particularly in those individuals who have been in contact with the virus. Follow-up testing with a molecular diagnostic assay should be considered to rule out infection in these individuals.
3. 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.
4. 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.
5. **Abnormal monospecific IgM results should be interpreted with caution**, as they may represent cross-reactivity with other human coronaviruses or Rheumatoid Factor IgM. Additionally, molecular testing for the diagnosis of acute COVID-19 may be indicated. See Results section for more details.
6. Not for screening of donated blood.

STORAGE AND HANDLING

1. Store all reagents at 2 – 8ºC when received. Avoid freezing reagents.
2. All reagents must be brought to room temperature (18 – 25ºC) for 30 minutes prior to use.
3. Avoid direct sunlight.
4. **Important**: When stored at 2 – 8 ºC, the 10X Wash Buffer may form crystals. The crystals must be dissolved prior to dilution of the 10X concentrate when only a portion of the concentrate is being diluted. If all the bottled contents are transferred at once to a 1-L graduated cylinder, be sure to rinse the bottle multiple times with water to dissolve and transfer any crystallized salts. When stored at 2 – 8 ºC, the 10X Wash Buffer is stable until kit expiration, the 1X Wash Buffer is stable for 8 weeks.

SPECIMEN REQUIREMENTS

Collection and Storage of Serum

A whole blood specimen should be obtained using accepted medical techniques to avoid hemolysis. The blood should be clotted, and the serum separated by centrifugation within 24 hrs of collection. Grossly hemolyzed, lipemic, or icteric serum is not acceptable since it may affect the results of the test. Serum may be stored at 2 - 8 °C for up to 7 days. If testing cannot be completed within 7 days of collection, the separated serum must be stored at –20°C. Do not use serum that has been thawed more than once or which has been heat inactivated. The performance of plasma samples has not been evaluated; therefore, plasma should not be used in the test.

PROCEDURE

Before starting the assay, read the product insert carefully. Instructions should be followed exactly as they appear in this kit insert to ensure valid results.

Materials Provided

EL-Anti-SARS-CoV-2 IgM/IgG Kit (Catalog No. 104-128)

1. **Two plates of antigen coated wells in 96-well plate format:** For single use only**!** All wells are coated with recombinant SARS-CoV-2 spike protein RBD antigen. Wells may be printed with the name of the antigen. The unused wells and the frame may be stored and used at a later date. They are returned to their desiccant-containing pouch, which is sealed and stored dry at 2 - 8°C until the expiration date.
2. **10X Wash Buffer, 1 x 100 mL:** 10X concentrated buffer with preservative.
3. **SARS-CoV-2 Specimen Diluent, 1 x 115 mL**: Buffer with bovine protein and preservative.
4. **SARS-CoV-2 IgM Calibrator, 1 x 1 mL:** Pre-diluted human serum with IgM antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
5. **SARS-CoV-2 IgG Calibrator, 1 x 1 mL:** Pre-diluted human serum with IgG antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
6. **SARS-CoV-2 IgM Positive Control, 1 x 1 mL:** Pre-diluted human serum containing IgM antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.

7. **SARS-CoV-2 IgG Positive Control, 1 x 1 mL:** Pre-diluted human serum containing IgG antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
8. **Negative Control, 1 x 0.35 mL:** Human serum without IgM or IgG antibodies against SARS-CoV-2 and preservative. See attached Data Sheet for performance characteristics.
9. **SARS-CoV-2 IgM Enzyme Conjugate, 1 x 15 mL:** Goat anti-human IgM ($Fc_5\mu$ specific) conjugated with horseradish peroxidase, with preservative in stabilizing buffer and blue dye.
10. **SARS-CoV-2 IgG Enzyme Conjugate, 1 x 15 mL:** Goat anti-human IgG (Fcγ specific) conjugated with horseradish peroxidase, with preservative in stabilizing buffer and green dye
11. **Chromogen, 1 x 27 mL:** 3,3'5,5' tetramethylbenzidine (TMB) in buffer with hydrogen peroxide.
12. **Stop Reagent, 1 x 27 mL:** 2 mol/L phosphoric acid.
13. **Re-sealable pouch**.

EL-Anti-SARS-CoV-2 IgM Kit (Catalog No. 104-129)

1. **One plates of antigen coated wells in 96-well plate format:** For single use only**!** All wells are coated with recombinant SARS-CoV-2 spike protein RBD antigen. Wells may be printed with the name of the antigen. The unused wells and the frame may be stored and used at a later date. They are returned to their desiccant-containing pouch, which is sealed and stored dry at 2 - 8°C until the expiration date.
2. **10X Wash Buffer, 1 x 100 mL:** 10X concentrated buffer with preservative.
3. **SARS-CoV-2 Specimen Diluent, 1 x 115 mL**: Buffer with bovine protein and preservative.
4. **SARS-CoV-2 IgM Calibrator, 1 x 1 mL:** Pre-diluted human serum with IgM antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
5. **SARS-CoV-2 IgM Positive Control, 1 x 1 mL:** Pre-diluted human serum containing IgM antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
6. **Negative Control, 1 x 0.35 mL:** Human serum without IgM or IgG antibodies against SARS-CoV-2 and preservative. See attached Data Sheet for performance characteristics.
7. **SARS-CoV-2 IgM Enzyme Conjugate, 1 x 15 mL:** Goat anti-human IgM ($Fc_5\mu$ specific) conjugated with horseradish peroxidase, with preservative in stabilizing buffer and blue dye.
8. **Chromogen, 1 x 27 mL:** 3,3'5,5' tetramethylbenzidine (TMB) in buffer with hydrogen peroxide.
9. **Stop Reagent, 1 x 27 mL:** 2 mol/L phosphoric acid.
10. **Re-sealable pouch**.

EL-Anti-SARS-CoV-2 IgG Kit (Catalog No. 104-130)

1. **One plate of antigen coated wells in 96-well plate format:** For single use only**!** All wells are coated with recombinant SARS-CoV-2 spike protein RBD antigen. Wells may be printed with the name of the antigen. The unused wells and the frame may be stored and used at a later date. They are returned to their desiccant-containing pouch, which is sealed and stored dry at 2 - 8°C until the expiration date.
2. **10X Wash Buffer, 1 x 100 mL:** 10X concentrated buffer with preservative.
3. **SARS-CoV-2 Specimen Diluent, 1 x 115 mL**: Buffer with bovine protein and preservative.

4. **SARS-CoV-2 IgG Calibrator, 1 x 1 mL:** Pre-diluted human serum with IgG antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
5. **SARS-CoV-2 IgG Positive Control, 1 x 1 mL:** Pre-diluted human serum containing IgG antibodies against SARS-CoV-2 and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
6. **Negative Control, 1 x 0.35 mL:** Human serum without IgM or IgG antibodies against SARS-CoV-2 and preservative. See attached Data Sheet for performance characteristics.
7. **SARS-CoV-2 IgG Enzyme Conjugate, 1 x 15 mL:** Goat anti-human IgG (Fcγ specific) conjugated with horseradish peroxidase, with preservative in stabilizing buffer and green dye
8. **Chromogen, 1 x 27 mL:** 3,3'5,5' tetramethylbenzidine (TMB) in buffer with hydrogen peroxide.
9. **Stop Reagent, 1 x 27 mL:** 2 mol/L phosphoric acid.
10. **Re-sealable pouch**.

Materials required but not provided

1. Calibrated precision micropipettes with disposable plastic tips that deliver 10 µL, 100 µL and 1 mL.
2. Calibrated adjustable multichannel pipettes (8- or 12-channel).
3. Disposable Pipette tips.
4. Microtubes, polypropylene (dilution tubes or cluster tubes) with a rack of 96-well format.
5. Timer.
6. Pipettes (1 mL, 5 mL, and 10 mL).
7. Pipette reagent reservoirs (to accommodate multichannel pipettes).
8. Deionized or distilled water.
9. Single (450 nm) or dual (450 nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA plate reader) for 96-well microtiter plates.
10. Clean wash bottle and automated plate washer (optional).

Reagent preparation:

1. Coated Wells

A suggested plate arrangement of wells is shown on the attached Data Sheet. The entire plate or strip (or strips) may be employed, or individual wells may be used as desired.

2. Wash Solution

The 10X Wash Buffer must be diluted 1:10 with deionized or distilled water prior to use. Prepare 1X Wash Buffer by pouring the contents of the 10X Wash Buffer into a clean one liter volumetric container. Rinse the bottle with deionized or distilled water to remove residual buffer and re dissolve any existing crystals. Add the rinse to the one-liter container. Add deionized or distilled water until a total volume of 1.0 L is reached; mix thoroughly. Diluted Wash Buffer is stable for 8 weeks at 2 - 8 °C.

3. Specimens

Specimens must be diluted 1:101 in the provided Specimen Diluent prior to being tested. Use high accuracy pipettes. For example, pipette 10 µL of serum into 1 mL of specimen diluent. Discard any unused diluted Specimens and Controls after the test procedure is completed.

4. Negative Control

The Negative Control must be diluted 1:101 in the provided Specimen Diluent prior to being tested. Use high accuracy pipettes. For example, pipette 10 µL of serum into 1 mL of specimen diluent. Discard any unused diluted Specimens and Controls after the test procedure is completed.

5. Calibrators and Positive Controls

The Calibrators and Positive Controls for each isotype are provided pre-diluted, ready to use. 100 µL of each should be pipetted directly into the appropriate wells on the test plate.

Assay Procedure

1. Allow all reagents and patient sera to equilibrate to room temperature prior to use (18-25°C). Plates should equilibrate to room temperature in their sealed foil pouch to prevent condensation.
2. Mark the position of the samples (i.e., Calibrator, Positive Control, Negative Control, and Specimens) on a worksheet, and arrange dilution tubes accordingly in a rack. A suggested plate arrangement is shown on the kit's Data Sheet
3. Determine the number of wells needed. The remaining unused wells should be returned and resealed in the pouch with desiccant for later use.
4. Dispense 1 mL of Specimen Diluent into each dilution tube.
5. Dilute all serum Specimens and the Negative Control 1:101 (e.g. add 10 µL of serum to 1 mL Specimen Diluent) and mix well. Do not dilute the Calibrator(s) or Positive Control(s).
6. Pipette 100 µL of the pre-diluted Calibrator(s) and Positive Control(s), and diluted Negative Control and Specimens into the appropriate wells. For best results pipette all materials within 5 minutes from the start of the assay. This step is facilitated using multichannel pipettes.
7. Incubate the plate for 60(± 5) minutes at room temperature (18 - 25 °C).
8. Aspirate or decant the contents of the wells and wash the plate 3 times with 300 µL of 1X Wash Buffer. An automated plate washer may be used for this step. Remove all residual liquid from the wells by inverting and blotting the plate on absorbent paper.
9. Immediately pipette 100 µL of IgM and/or IgG Enzyme Conjugate(s) into the appropriate wells, according to the plate map.
10. Incubate plate(s) for 30(± 5) minutes at room temperature (18 - 25 °C).
11. Aspirate or decant Enzyme Conjugate from all wells and wash the plate as in Step 8 above.
12. Immediately dispense 100 µL of Chromogen into each well. Incubate the plate(s) for 15(±1) minutes at room temperature (18 - 25 °C).
13. Pipette 100 µL of Stop Reagent into each well and mix by gently tapping the side of the plate. The blue color changes to yellow.
14. Determine the absorbance of each well at 450 nm using a single or dual wavelength spectrophotometer (ELISA plate reader). Absorbance values should be read within 30 minutes of completing the assay. For a dual wavelength spectrophotometer, set test wavelength at 450 nm with the reference between 620 and 690 nm.

Procedural Notes

1. Storage
Place unused strips in the open metallized pouch (with desiccant) for light protection and place this assembly into the provided re-sealable pouch and store at 2 - 8 °C.

2. Pipetting
To avoid cross-contamination and sample carryover, pipette the Calibrator, Positive Control, Negative Control, and Specimens using separate pipette tips. A multi-channel pipette may be used to pipette the Enzyme Conjugates, Wash Solution, Chromogen and Stop Reagent.

3. Washing
Each column of wells may be washed using a multi-channel pipette. The wells may be aspirated using an appropriate vacuum apparatus, fitted with a Pasteur pipette, or their contents may be dumped into a disposal container. Alternatively, commercial semi-automated washing systems may be used. When using either washing technique, the plate should be inverted and blotted against absorbent paper after the last wash. Use reagent grade water only (CAP type 1 or USP grade) for preparing the 1X Wash Buffer.

4. Measurement of Absorbance Values
Absorbance values should be measured within 30 minutes after completion of the assay.

RESULTS

Calculation of Results
Most ELISA readers are computer compatible and data may be calculated with the help of computer programs. Check periodically that the program chosen yields the same results as obtained by manual calculations. Antibody activity is calculated as follows:

$$\text{Conversion Factor} = \frac{\text{Unit value of SARS-CoV-2 Calibrator}}{\text{Absorbance (OD) value of SARS-CoV-2 Calibrator}}$$

Antibody Units in Specimen = Conversion Factor x Absorbance value of Specimen

Interpretation

Assessment of **EL-Anti-SARS-CoV-2** assay results should be performed after the calibrator, positive, and negative controls have been examined and determined to be valid and acceptable according to the ranges listed on the data sheet. If the controls are not valid, the patient results cannot be interpreted.

Reference range:
Negative: ≤ 50 Units
Abnormal: > 50 Units

Test Result Interpretation			
	Analytes		
Outcome	IgM	IgG	Interpretation
1	≤ 50 Units	≤ 50 Units	Specimen within normal range
2	>50 Units	≤ 50 Units	Abnormal IgM levels present
3	≤ 50 Units	>50 Units	Abnormal IgG levels present
4	>50 Units	>50 Units	Abnormal IgM and IgG levels present

The stated ranges for IgM and IgG isotypes are suggested values only. The reference range should be validated by each laboratory to reflect the characteristics of the population they serve.

Monospecific IgM abnormal results should be interpreted with caution. They may represent cross-reactivity with elevated RF IgM or antibodies against other betacoronaviruses. This result also suggests the need for molecular testing for diagnosis of possible acute COVID-19 and follow-up serology in one to two weeks, as the absence of IgG may indicate an early phase of the illness[7].

During test development blood bank normal specimens drawn prior to the COVID-19 outbreak were observed to measure up to 100 Units for IgM. This observation is likely explained by the highly conserved genome (>99.9%) of SARS-CoV-2 in comparison to other viruses of the betacoronavirus genera[6]. Several betacoronaviruses (CoV strains SARS, MERS, OC43, 229E) have circulated prior to the outbreak of COVID-19, have been identified as causes of other illnesses, and have likely generated immunity in the general population[7]. Additionally, serum samples with elevated levels of RF IgM have been shown to generate false-positive results on SARS-CoV-2 immunoassays[5].

QUALITY CONTROL

1. Calibrators

The **EL-Anti-SARS-CoV-2** IgM and IgG calibrators must be run with each assay and the absorbance values must fall within the range listed on the Data Sheet. If the values are not in agreement with those on the Data Sheet, the assay is not valid, and the results should not be reported.

2. Positive and Negative Controls

Positive and Negative Controls should be run in each assay. The Positive and Negative Control unit values should fall within the ranges provided on the enclosed Data Sheet. If the values are not in agreement with those on the Data Sheet, the assay is not valid, and the results should not be reported.

LIMITATIONS OF THE PROCEDURE

1. The Positive Control and the Calibrator for a specific antibody may contain other antibodies, i.e. they may not be monospecific.
2. The **TheraTest EL-Anti-SARS-CoV-2** assay(s) should not be performed on grossly hemolyzed, lipemic, icteric or microbially contaminated samples. The effect of hemolysis, lipemia, and icterus has not been evaluated with this assay.
3. This method has been tested using serum samples only. The performance using other types of specimens has not been determined.
4. Diagnosis should not be made solely on the basis of a positive test result. The results must be interpreted in conjunction with all clinical information and laboratory data available to the physician (i.e. history, physical exam, and other diagnostic procedures).
5. This assay has not been evaluated on a pediatric population.
6. If the absorbance value of the Specimen exceeds the linear range of the reader, the result should be reported as > Units (of the upper limit of the linear range). If endpoint result is desired, the Specimen should be pre-diluted (example: 1:10) with the provided Specimen Diluent, and the Specimen should be retested. The retest result should be multiplied by the pre-assay dilution factor (for example, if the Specimen was pre-diluted 1:10, the units obtained should be multiplied by 10). There is no linear relationship between the dilution factor and the obtained unit values.
7. As no qualified international reference serum exists for antibodies against SARS- CoV-2 the calibration is performed in arbitrary units which are a relative measure of the concentration of antibodies.

EXPECTED VALUES

The **TheraTest EL-Anti-SARS-CoV-2** assay(s) was designed to qualitatively assess the presence of anti-SARS-CoV-2 IgM and IgG antibodies. Immunocompetent patients diagnosed with COVID-19 are expected to develop antibodies to the SARS-CoV-2 virus. Early studies suggest the presence of IgM and IgG antibodies is detectable at 3 – 5 days and 2 weeks from the onset of symptoms, respectively [7]. While the duration of the detectability of these antibodies remains to be studied, IgM antibodies may be present for two months, and IgG antibodies may be present for three years or longer, based on serology studies of the related SARS- and MERS-CoV viruses[7]. The production of antibodies against SARS-CoV-2 is part of the adaptive immune response to the virus, however the levels of antibodies required to confer immunity have not yet been determined[4]. Until the protective value of these antibodies has been established, positive results on this test should be interpreted as suggestive of possible exposure, rather than an indication of immunity.

PERFORMANCE CHARACTERISTICS

The clinical sensitivity and specificity of this assay have been found to meet or exceed the minimum performance requirements put forth by the FDA's Center for Devices and Radiological Health in the document "Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)", published in May 2020.

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is exceptionally limited at present).

Comparative studies

A total of 266 samples were tested on the **TheraTest EL-Anti-SARS-CoV-2** assays for comparative analysis. This testing included 100 blood bank normal specimens collected prior to May 21st 2019, serum samples from 42 patients with RT PCR confirmed SARS-CoV-2 infection, and 124 specimens for assessment of other infectious and auto-immune illness cross-reactivity. Details of the normal population demographics, cross-reactivity specimens, and results of comparative testing are discussed in the tables below.

Blood Bank Normals - Demographics				
White/Caucasian	69		Male	50
Hispanic	15		Female	50
African American	12		Age Range	19-71
Asian	4		Average Age	39

TheraTest EL-Anti-SARS-CoV-2 IgM				
		RT PCR		
		Positive	Negative	Total
TTL SARS-CoV-2	Positive	30	10	40
	Negative	12	214	226
	Total	42	224	266
Clinical Agreement				
		Results	Required	95% CI
Percent Positive		71.4%*	≥ 70%	55.2-83.8%
Percent Negative		95.5%	≥ 95%	91.7-97.7%
Overall		91.7%		87.6-94.6%

*Studies of the immune response to the SARS-CoV outbreak in 2003 have noted waning IgM seropositivity over time. IgM seropositivity peaked at 76% of patients from days 21-30 post symptom onset. Over 35% of patients were found to be seronegative for IgM before and after this peak window[7]. This is a likely explanation for IgM seronegativity in patients with reactive IgG antibodies.

TheraTest EL-Anti-SARS-CoV-2 IgG				
		RT PCR		
		Positive	Negative	Total
TTL SARS-CoV-2	Positive	39	9	48
	Negative	3	215	218
	Total	42	224	266
Clinical Agreement				
		Results	Required	95% CI
Percent Positive		92.9%	≥ 90%	87.6-94.6%
Percent Negative		96.0%	≥ 95%	92.3-98.0%
Overall		95.5%		92.1-97.5%

Cross Reactivity

One hundred twenty four specimens of known seropositivity for infectious or autoimmune illnesses were tested for cross-reactivity. The disease state and quantity of specimens tested are listed below, and the testing results were included in the clinical agreement analysis above.

Cross-Reactivity			
Analyte	Count	Analyte	Count
Cytomegalovirus	5	RNP/Sm	5
Herpes simplex virus 1	5	SSA	5
Hepatitis B	5	SSB	5
Hepatitis C	5	Chromatin	5
Rubella	5	SCL-70	5
Varicella Zoster	5	Centromere	5
HIV	5	Histone	5
Influenza A.	5	Jo-1	5
ssDNA	5	Ribosome-P	5
dsDNA	5	RA Panel*	24
Sm	5	**Total**	**124**

* RA Panel includes specimens highly reactive for RF IgM, IgG, IgA, and CCP/2

Class Specificity

Class specificity of enzyme conjugate solution is assessed for each lot according the TheraTest Laboratories standard Quality Control protocol.

TROUBLESHOOTING

Problem	Possible Causes	Solution
Control values out of range.	1. Incorrect temperature, timing or pipetting; reagents not mixed.	1. Check that temperature was correct. Check that time was correct. See "Poor Precision" (below) No. 2-4. Repeat test.
	2. Cross-contamination of controls.	2. Pipette carefully.
	3. Improper dilution.	3. Repeat test.
	4. Optical pathway not clean.	4. Check for moisture or dirt. Wipe bottom and reread.
	5.Wavelength of filter incorrect.	5. Change filter to 450 ± 5 nm.
All test results negative.	1. One or more reagents not added, or added in wrong sequence.	1. Recheck procedure. Check for unused solutions. Repeat test.
	2. Improper dilution of wash buffer.	2. Repeat test.
	3. Antigen coated plate inactive.	3. Check for obvious moisture in unused wells. Rerun test with controls only for activity.
All test results yellow.	1. Contaminated chromogen.	1. Check absorbance of unused chromogen.
Scattered false positives	2. Contaminated buffers/reagents.	2. Check all solutions for turbidity.
	3. 1X Wash Buffer contaminated.	3. Use clean container. Check quality of water used to prepare buffer.
	4. Improper dilution of serum.	4. Repeat test.
	5. Contaminated pipette	5. Use plugged tips for chromogen
Poor precision.	1. Pipettor delivery CV greater than 5%.	1. Check calibration of pipettor. Use reproducible technique.
	2. Serum or reagents not mixed sufficiently; reagents not at room temperature prior to addition.	2. Mix all reagents gently but thoroughly and equilibrate to room temperature.
	3. Reagent addition taking too long; inconsistency in timing intervals, air bubbles.	3. Develop consistent uniform technique and avoid splashing or use multi-channel device or auto-dispenser to decrease time.
	4. Air currents blowing over plate during incubations.	4. Cover plate or place in chamber.
	5. Optical pathway not clean.	5. Wipe bottom of plate with soft tissue. Check instrument light source and detector for dirt.
	6. Instrument not equilibrated before readings were taken.	6. Check instrument manual for warm up procedure.
	7. Washing not consistent; trapped bubbles; liquid left in wells at end of wash cycle.	7. Use only acceptable washing devices. Lengthen timing delay on washing devices. Check that all wells are filled.
	8. Improper pipetting.	8. Avoid air bubbles in pipette tips.

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(GB)(USA)(CDN) Expiry date (D)(A)(B)(CH) Verfallsdatum (F)(B)(CH)(CDN) Date de péremption (I)(CH) Data di scadenza (E) Fecha de caducidad (P) Data de validade (NL) Uiterste gebruiksdatum (DK) Udløbsdato (S) Utgångsdatum

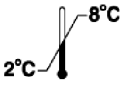
(GB)(USA)(CDN) Consult instructions for use (D)(A)(B)(CH) Bitte Gebrauchsanweisung einsehen (F)(B)(CH)(CDN) Consultez la notice d'utilisation (I)(CH) Consultare le istruzioni per l'uso (E) Consulte las instrucciones de utilización (P) Consulte as instruções de utilização (NL) Raadpleeg de gebruikaanwijzing (DK) Se brugsanvisningen (S) Läs anvisningarna före användning

(GB)(USA)(CDN) In-vitroDiagnostic Medical Device (For In-vitroDiagnostic Use) (D)(A)(B)(CH) Medizinisches In-vitro-Diagnostikum (zur In-vitro-Diagnostik) (F)(B)(CH)(CDN) Dispositif médical de diagnostic in-vitro(Pour usage diagnostique in vitro) (I)(CH) Dispositivo medico per diagnostica in-vitro(per uso diagnostico in vitro) (E) Dispositivo médico de diagnóstico in-vitro(para uso diagnóstico in vitro) (P) Dispositivo médico para diagnóstico in-vitro(Para utilização de diagnóstico "in vitro") (NL) Medisch hulpmiddel voor diagnostiek in-vitro(Voor diagnostisch gebruik in vitro) (DK) Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in-vitrodiagnostisk anvendelse) (S) Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk)

(GB)(USA)(CDN) Lot / Batch Number (D)(A)(B)(CH) Charge / Chargennummer (F)(B)(CH)(CDN) Lot / Code du lot (I)(CH) Lotto / Numero lotto (E) Lote / Código de lote (P) Lote / Código do lote (NL) Lot-/Partijnummer (DK) Lot / Batchkode (S) lot / Satskod

(GB)(USA)(CDN) Manufactured by (D)(A)(B)(CH) Hergestellt von (F)(B)(CH)(CDN) Fabriqué par (I)(CH) Prodotto da (E) Fabricado por (P) Fabricado por (NL) Vervaardigd door (DK) Fabrikation af (S) Tillverkad av

(GB)(USA)(CDN) Catalogue Number (D)(A)(B)(CH) Bestell-Nummer (F)(B)(CH)(CDN) Numéro de référence (I)(CH) Numero di riferimento (E) Número de referencia (P) Número de referência (NL) Referentienummer (DK) Referencenummer (S) Katalognummer



(GB)(USA)(CDN) Store at between (D)(A)(B)(CH) Lagerung bei zwischen (F)(B)(CH)(CDN) Conserver à entre (I)(CH) Conservare a tra (E) Conservar a temp. entre (P) Armazene a entre (NL) Bewaar bij tussen (DK) Opbevares mellem (S) Förvaras vid

(GB)(USA)(CDN) Contains sufficient for x tests (D)(A)(B)(CH) Inhalt ausreichend für x Tests (F)(B)(CH)(CDN) Contient suffisant pour x tests (I)(CH) Contenuto sufficiente per x test (E) Contiene suficiente para x pruebas (P) Contém suficiente para x testes (NL) Bevat voldoende voor x bepalingen (DK) Indeholder tilstrækkeligt til x prøver (S) Innehållet räcker till x analyser

(GB)(USA)(CDN) Caution; Consult accompanying documents. (D)(A)(B)(CH) Achtung. begleitdokumente beathten. (F)(B)(CH)(CDN) Attention, consulter les documents joints. (I)(CH) Attenzione, consultare la documentazione allegata. (E) Precaucion, consultar la documentacion adjunta. (P) Cuidado, consulte a documentação fornecida. (NL) Let op, raadpleeg bijgeleverde documenpen. (DK) Forsigtig, Læs ledsagende dokunenter. (S) Forsiktig, se vedlagt dokumentasjon.

Abbreviated Test Procedure

1. **Dilute Negative Control and Specimens 1:101 with Specimen Diluent.**
2. **Pipette 100 µL of Calibrator(s), Positive Control(s), and diluted Negative Control and Specimens into appropriate wells (see Data Sheet for configuration).**
3. **Incubate for 60 (± 5) minutes at room temperature (18º - 25ºC).**
4. **Wash the wells three times with 1X Wash Buffer.**
5. **Add 100 µL of the IgM and/or IgG Enzyme Conjugate(s) into appropriate wells according to the plate map.**
6. **Incubate for 30 (± 5) minutes at room temperature (18º - 25ºC).**
7. **Wash the wells three times with 1X Wash Buffer.**
8. **Add 100 µL of Chromogen into each well.**
9. **Incubate for 15 (± 1) minutes at room temperature (18º- 25ºC).**
10. **Add 100 µL of Stop Reagent into each well.**
11. **Read the absorbance at 450 nm (reference wavelength 620-690 nm) within 30 minutes.**

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