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Changes: § 5
Deletions: §

# LIAISON® SARS-CoV-2 TrimericS IgG ( REF 311510)

## 1. INTENDED USE

The LIAISON® SARS-CoV-2 TrimericS IgG assay uses chemiluminescence immunoassay (CLIA) technology for the quantitative determination of anti-trimeric spike protein specific IgG antibodies to SARS-CoV-2 in human serum or plasma samples. The assay is intended as an aid in the diagnosis of CoVID-19 and to support the study of the immune status of infected patients and to assess IgG response against SARS-CoV-2 in vaccine recipients, by providing an indication of the presence of neutralizing IgG antibodies against SARS-CoV-2. Results from the LIAISON® SARS-CoV-2 TrimericS IgG assay should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform about infection status.

The test has to be performed on the LIAISON® Analyzer Family*.

## 2. SUMMARY AND EXPLANATION OF THE TEST

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. At the end of December 2019, Chinese public health authorities reported several cases of acute respiratory syndrome in Wuhan City, Hubei province, China. The initial outbreak in Wuhan spread rapidly, affecting other parts of China. Cases were then detected in several other countries. Since late February, the majority of cases reported are from outside China, with an increasing majority of these reported from EU/EEA countries and the US. The Director General of the World Health Organization declared COVID-19 a global pandemic on 11 March 2020.(1,2).
The causative virus of the COVID -19 is Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). It is a new strain of coronavirus that has not been previously identified in humans. It spreads primarily through contact with an infected person through respiratory droplets generated when a person coughs or sneezes, or through droplets of saliva or discharge from the nose.
Infection with SARS-CoV-2 can cause mild symptoms including a runny nose, sore throat, cough and fever. However, it can be more severe for some people and can lead to pneumonia or breathing difficulties. The elderly and people with pre-existing medical conditions (such as, diabetes and heart disease) appear to be more vulnerable to becoming severely ill with the virus. Based on previous studies on SARS, an incubation period from three to fourteen days after onset of symptoms may be expected. (3)
The presence of IgG antibodies to SARS-CoV-2 is indicative of an immune response to infection; however, it is unknown whether the presence of IgG antibodies to SARS-CoV-2 confers protective immunity or for how long after infection IgG antibodies will remain detected. Patients can remain infectious in the presence of IgG if specimens are obtained during acute infection (4).
Currently, molecular testing is available using reverse transcription-polymerase chain reaction (RT-PCR) for detecting viral RNA for early identification of SARS-CoV-2.
The coronavirus spike (S) glycoprotein is a class I viral fusion protein on the outer envelope of the virion that plays a critical role in viral infection by recognizing host cell receptors and mediating fusion of the viral and cellular membranes. The transmembrane S glycoprotein which forms homotrimers protruding from the viral surface is the main target of neutralizing antibodies and the primary target for vaccine design (5).

## 3. PRINCIPLE OF THE PROCEDURE

The LIAISON® SARS-CoV-2 TrimericS IgG assay is an indirect chemiluminescence immunoassay (CLIA) for the detection of IgG antibodies to SARS-CoV-2 in human serum and plasma samples. The principal components of the test are magnetic particles (solid phase) coated with recombinant trimeric SARS-CoV-2 spike protein and a conjugate reagent containing an anti-human IgG mouse monoclonal antibody linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, SARS-CoV-2 IgG antibodies present in calibrators, samples or controls bind to the solid phase. Unbound material is then removed with a wash cycle. During the second incubation, the antibody conjugate reacts with antibodies to SARS-CoV-2 already bound to the solid phase. Excess antibody conjugate is then removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of antibodies to SARS-CoV-2 present in calibrators, samples or controls.

*(LIAISON® XL and LIAISON® XS)

## 4. MATERIALS PROVIDED

**Reagent Integral**

| | | |
|---|---|---|
| Magnetic Particles (2.6 mL) | SORB | Magnetic particles coated with recombinant trimeric SARS-CoV-2 spike protein in phosphate buffer containing BSA and < 0.1% sodium azide |
| Calibrator 1 (1.2 mL) | CAL 1 | Human serum/plasma containing low SARS-CoV-2 IgG antibody levels, stabilized in TRIS buffer, 0.2% Proclin® 300, preservatives. |
| Calibrator 2 (1.2 mL) | CAL 2 | Human serum/plasma containing high SARS-CoV-2 IgG antibody levels, stabilized in TRIS buffer, 0.2% Proclin® 300, preservatives. |
| Specimen Diluent (2 x 29 mL) | DIL SPE | Phosphate buffer containing BSA, non-specific recombinant protein (produced in *E. coli*), detergent, EDTA, 0.2% ProClin® 300, preservatives. |
| Conjugate (25 mL) | CONJ | Mouse monoclonal antibody to human IgG conjugated to an isoluminol derivative in a phosphate buffer containing BSA, surfactant, 0.2% ProClin® 300, preservatives. |
| Number of Tests | | 110 |

ProClin® is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral.

The calibrator concentrations expressed as Binding Antibody Units (BAU)/mL are referenced to the First WHO International Standard for anti-SARS-CoV-2 Immunoglobulin (20/136) (6).

**Materials required but not provided (system related)**

| LIAISON® XL Analyzer | LIAISON® XS Analyzer |
|---|---|
| LIAISON® Wash/System Liquid (REF 319100)<br>- | LIAISON® EASY Wash Buffer (REF 319301)<br>LIAISON® EASY System Liquid (REF 319302) |
| LIAISON® XL Waste Bags (REF X0025) | LIAISON® EASY Waste (REF X0054) |
| LIAISON® XL Cuvettes (REF X0016) | LIAISON® Cuvettes on Tray (REF X0053) |
| LIAISON® XL Starter Kit (REF 319200) or<br>LIAISON® EASY Starter Kit (REF 319300) | -<br>LIAISON® EASY Starter Kit (REF 319300) |
| LIAISON® XL Disposable Tips (REF X0015) or<br>LIAISON® Disposable Tips (REF X0055) | LIAISON® Disposable Tips (REF X0055) |
| - | LIAISON® EASY Cleaning Tool (REF 310996) |

**Additional required materials:**
LIAISON® SARS-CoV-2 TrimericS IgG Control Set (REF 311511)

**Additional recommended materials**
LIAISON® TrimericS IgG Diluent Accessory (REF 311512)

## 5. WARNINGS AND PRECAUTIONS

**FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.**

**General Safety:**

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.

**Chemical Hazard and Safety Information**: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and applicable European Union directives (see Material Safety Data Sheet for additional information).

**Reagents Containing Human Source Material:**
Warning – Treat as potentially infectious. Each serum/plasma donor unit used in the preparation of this product has been tested by an U.S. FDA approved method and found non-reactive for the presence of the antibody to Human Immunodeficiency Virus 1 and 2 (HIV 1/2), the Hepatitis B surface antigen (HBsAg), and the antibody to Hepatitis C (HCV). While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source diseases for which there is no approved test. Because no known test method can offer complete assurance that HIV, Hepatitis B Virus (HBV) and HCV or other infectious agents are absent, all products containing human source material should be handled following universal precautions; and as applicable in accordance with good laboratory practices as described in the Centers for Disease Control and the National Institutes of Health current manual, Biosafety in Microbiological and Biomedical Laboratories (BMBL); or the World Health Organization current edition, Laboratory Biosafety Manual.

**GHS/CLP:**

| | Sodium Azide | ProClin® |
|---|---|---|
| CAS No.: | 26628-22-8 | 55965-84-9 |
| Reagents: | SORB | CAL 1<br>CAL 2<br>DIL SPE<br>CONJ |
| Classification: | None required | Skin sensitization, Category 1<br>Aquatic Chronic, Category 3 |
| Signal Word: | None required | Warning |
| Pictogram: | None required |  <br>GHS07 – Exclamation mark |
| Hazard Statements: | None required | H317 – May cause an allergic skin reaction.<br>H412 – Harmful to aquatic life with long lasting effects. |
| Precautionary Statements: | None required | P261 – Avoid breathing dust, fumes, gas, mist, vapors or spray.<br>P272 – Contaminated work clothing should not be allowed out of the workplace.<br>P273 – Avoid release to the environment.<br>P280 – Wear protective gloves, protective clothing, eye protection, and face protection. |

**REAGENTS CONTAINING SODIUM AZIDE:** Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

## 6. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

### 6.1 Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended.
- Repeat as necessary until the magnetic particles are completely resuspended.
- After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

### 6.2 Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

- Visually inspect the reagents to ensure there is no foaming present before using the integral. If foam is present after re-suspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

### 6.3 Loading of integral into the reagent area

**LIAISON® XL and LIAISON® XS Analyzer**

- LIAISON® XL and LIAISON® XS Analyzers are equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a Reagent Integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
    a. Insert the reagent integral into the dedicated slot.
    b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

## 7. STORAGE AND STABILITY OF THE REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate re-suspension of magnetic particles. When the Reagent Integral is stored unopened the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral should not be used past the expiration date indicated on the kit and Reagent Integral labels. After removing seals, the Reagent Integral may be returned to the kit box and stored upright at 2-8°C or stored on board the Analyzer for up to 4 weeks. Undue exposure to light should be avoided.

## 8. SPECIMEN COLLECTION AND PREPARATION

Human serum and plasma ( lithium heparin and potassium EDTA) or serum separator tubes may be used. Blood should be collected aseptically by venipuncture. Serum samples should be allowed to clot. Centrifuge samples and separate serum from the clot or plasma from the cells as soon as possible. No additives or preservatives are required to maintain integrity of the sample. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. Samples are stable at room temperature for up to 48 hours. If the assay is performed within 21 days of sample collection, the samples should be kept at 2-8°C; otherwise they should be stored frozen at -20°C or below for up to 2 months. If samples are stored frozen, mix thawed samples well before testing. Samples may be frozen-thawed 3 times. Self-defrosting freezers are not recommended for sample storage.

It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

The minimum specimen volume required for a single determination is 160 µL. [10 µL specimen for testing + 150 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)].

## 9. CALIBRATION

Individual LIAISON® SARS-CoV-2 TrimericS IgG Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows 5 calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- The previous calibration was performed more than 4 weeks prior.
- Quality Control results are out of the acceptable range.
- The Analyzer has been serviced.

Refer to the analyzer operator's manual for calibration instructions.

**Measuring range:** The LIAISON® SARS-CoV-2 TrimericS IgG assay measures between 4.81 and 2080 BAU/mL. The lowest reportable value is 4.81 BAU/mL. Values below 4.81 BAU/mL should be reported as < 4.81 BAU/mL.

Specimens containing high levels of anti-trimeric spike protein specific IgG antibodies to SARS-CoV-2 above the assay measuring range (> 2080 BAU/mL) may be automatically diluted using LIAISON® TrimericS IgG Diluent Accessory ( REF 311512) loaded into the ancillary reagent area. The required dilution factor is 1:20. It is the responsibility of the individual laboratory to conduct studies to determine validity of dilutions other than 1:20.

## 10. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Analyzer.

**LIAISON® XL and LIAISON® XS Analyzer:** Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral: contact your local DiaSorin technical support for instruction.

For details, refer to the analyzer operator's manual.

The analyzer operations are as follows:

1. Dispense sample, calibrator or control, magnetic particles and specimen diluent into reaction cuvette.
2. Incubate
3. Wash with Wash/System liquid
4. Dispense conjugate into reaction cuvette.
5. Incubate
6. Wash with Wash/System liquid
7. Add the Starter Reagents and measure the light emitted.

## 11. QUALITY CONTROL

Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3 and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® SARS-CoV-2 TrimericS IgG controls are intended to monitor for substantial reagent failure. LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

## 12. INTERPRETATION OF RESULTS

The Analyzer automatically calculates the SARS-CoV-2 IgG antibody levels expressed as Binding Antibody Units (BAU/mL) and grades the results. For details, refer to the analyzer operator's manual.

Patient results should be interpreted as follows:

| BAU/mL | Results | Interpretation |
|---|---|---|
| < 33.8 | Negative | A negative result may indicate the absence or a very low level of IgG antibodies to the pathogen. The test could score negative in infected patients during the incubation period and in the early stages of infection. |
| ≥ 33.8 | Positive | A positive result indicates the presence of IgG antibodies to SARS-CoV-2 and generally indicates exposure to SARS-CoV-2. |

Test results are reported as positive or negative along with a numeric value for quantitative measurement. However, diagnosis of SARS-CoV-2 infection should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings, patient history, and always in association with medical judgment.

## 13. LIMITATIONS OF THE PROCEDURE

1. For in vitro diagnostic use.
2. For professional use Only
3. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
4. Bacterial contamination of samples may affect the test results.
5. Detection of IgG antibodies against SARS-CoV-2 at present is not yet established to determine long term immunity to the virus or to protect the patient against re-infection by the virus.
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. The results obtained with this test should only be interpreted in conjunction with clinical findings, and the results from other laboratory tests and evaluations. This is especially important if the patient has had recent exposure to COVID-19, or clinical presentation indicates that COVID-19 is likely and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. In this case, direct testing for the SARS-CoV-2 virus (e.g. PCR testing) should be considered.
8. This test should not be used for screening of donated blood.
9. Integrals may not be exchanged between analyzer types (LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.
10. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON® XL or LIAISON® XS).
11. This device has not been tested with samples positive for SARS-CoV-1 and MERS-CoV antibodies.
12. Patient antibodies to SARS-CoV-2 are heterogeneous, which may lead to a non-linear dilution response.

## 14. SPECIFIC PERFORMANCE CHARACTERISTICS

### 14.1 Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, hemolysis, effects of sample treatment), or cross-reactive antibodies.

**Cross-reactions**.

The cross-reactivity study for the LIAISON® SARS-CoV-2 TrimericS IgG assay was designed to evaluate potential cross-reactivity to antibodies to other viruses that may cause symptoms similar to SARS-CoV-2 infection, to other organisms that may cause infectious diseases, as well as to other conditions that may result in atypical immune system activity. Two (2) specimens out of 396 assessed specimens resulted Positive with the LIAISON® SARS-CoV-2 TrimericS IgG assay. The results are summarized in the following tables.

| Non-SARS Human Coronavirus Condition | Number of tested samples | Positive results |
|---|---|---|
| Anti-Human CoV 229E | 6 | 0 |
| Anti-Human CoV OC43 | 7 | 0 |
| Anti-Human CoV HKU1 | 7 | 0 |
| Anti-Human CoV NL63 | 11 | 0 |
| Total | 31 | 0 |

| Condition | Number of tested samples | Positive results |
|---|---|---|
| Anti-EBV IgG | 10 | 0 |
| Anti-CMV IgG | 14 | 0 |
| Anti-Rubella IgG | 10 | 0 |
| Anti-Parvovirus B19 IgG | 13 | 0 |
| Anti-Borrelia burgdorferi | 10 | 0 |
| Anti-HSV-1/2 IgG | 20 | 0 |
| Anti-VZV IgG | 10 | 0 |
| Anti-HCV | 10 | 0 |
| Anti-HBV | 60 | 1 |
| Anti-HIV | 60 | 0 |
| Anti-West Nile Virus | 15 | 0 |
| Rheumatoid factor | 16 | 0 |
| HAMA | 27 | 0 |
| Anti-nuclear autoantibodies (ANA) | 29 | 0 |
| Anti-Mycoplasma pneumoniae IgG | 4 | 0 |
| Anti-Influenza A | 10 | 0 |
| Anti-Influenza B | 9 | 0 |
| Anti-Influenza A/B | 9 | 0 |
| Anti-respiratory syncytial virus A | 5 | 0 |
| Anti-respiratory syncytial virus B | 1 | 0 |
| Anti-rhinovirus/Enterovirus | 5 | 0 |
| Anti-human metapneumovirus | 5 | 0 |
| Anti-human parainfluenza virus | 4 | 1 |
| Anti-Adenovirus | 9 | 0 |
| Total | 365 | 2 |

**Interference**.

Controlled studies of potentially interfering substances showed no interference to each substance listed below in the LIAISON® SARS-CoV-2 TrimericS IgG assay, at the indicated concentration.

| Substances | Tested concentrations |
|---|---|
| Triglycerides | 3000 mg/dL |
| Hemoglobin | 1000 mg/dL |
| Unconjugated bilirubin | 40 mg/dL |
| Conjugated bilirubin | 40 mg/dL |
| Cholesterol total | 400 mg/dL |
| Human Serum Albumin | 6 g/dL |
| Biotin | 3500 ng/mL |
| Acetaminophen | 500 µg/mL |
| Ibuprofen | 500 µg/mL |

### 14.2 Precision

**LIAISON® XL**

A 5 day precision study was performed by using a coded panel of 6 serum samples prepared by blending samples as necessary to obtain negative, low positive and moderate positive samples. Kit Controls were also included in the study. The panel samples and kit controls were tested with 2 lots of LIAISON® SARS-CoV-2 TrimericS IgG assay in 3 replicates per run, 2 runs per day for 5 operating days at 2 testing sites on 2 LIAISON® XL Analyzers. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

**LIAISON® SARS-CoV-2 TrimericS IgG Precision – Two Lots Combined, Multi-Site**

| Sample Number | n | Mean | Within run | | Between run | | Between day | | Overall | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | CV | SD | CV | SD | CV | SD | CV |
| Neg Kit Control* | 60 | 665 | 34 | 5.1% | 40 | 6.0% | 28 | 4.2% | 60 | 9.0% |
| Pos Kit Control | 60 | 90.58 | 1.617 | 1.8% | 0.595 | 0.7% | 3.039 | 3.4% | 3.493 | 3.9% |
| Sample 1 | 60 | 13.00 | 0.253 | 1.9% | 0.162 | 1.2% | 0.38 | 2.9% | 0.514 | 4.0% |
| Sample 2 | 60 | 33.12 | 0.519 | 1.6% | 0.547 | 1.7% | 0.918 | 2.8% | 1.188 | 3.6% |
| Sample 3 | 60 | 37.16 | 0.638 | 1.7% | 0.000 | 0.0% | 1.867 | 5.0% | 2.137 | 5.7% |
| Sample 4 | 60 | 637.6 | 20.549 | 3.2% | 5.364 | 0.8% | 26.606 | 4.2% | 36.896 | 5.8% |
| Sample 5 | 60 | 1307 | 58.054 | 4.4% | 0.000 | 0.0% | 27.734 | 2.1% | 70.602 | 5.4% |
| Sample 6 | 60 | 1536 | 55.926 | 3.6% | 34.624 | 2.3% | 0.000 | 0.0% | 70.304 | 4.6% |

* BAU/mL values for the Negative Controls fall below the assay range and were evaluated based on RLUs instead of BAU/mL.

**LIAISON® XS**

A 5 day precision study was performed by using a coded panel of 6 serum samples prepared by blending samples as necessary to obtain negative, low positive and moderate positive samples. Kit Controls were also included in the study. The panel samples and kit controls were tested with 1 lot of LIAISON® SARS-CoV-2 TrimericS IgG assay in 6 replicates per run, 1 run per day for 5 operating days at 1 testing site on 3 LIAISON® XS Analyzers. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

**LIAISON® SARS-CoV-2 TrimericS IgG Precision – Three Analyzers Combined**

| Sample Number | n | Mean | Within run | | Between run | | Total within Analyzer | | Between Analyzer | | Overall | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | CV | SD | CV | SD | CV | SD | CV | SD | CV |
| Neg Kit Control* | 90 | 454 | 20.887 | 4.6% | 8.628 | 1.9% | 20.928 | 4.6% | 39.369 | 8.7% | 44.419 | 9.8% |
| Pos Kit Control | 90 | 94.2 | 1.713 | 1.8% | 1.053 | 1.1% | 1.885 | 2.0% | 7.312 | 7.8% | 7.537 | 8.0% |
| Sample 1 | 90 | 11.8 | 0.152 | 1.3% | 0.229 | 1.9% | 0.268 | 2.3% | 0.744 | 6.3% | 0.784 | 6.7% |
| Sample 2 | 90 | 33.8 | 0.350 | 1.0% | 0.427 | 1.3% | 0.533 | 1.6% | 1.692 | 5.0% | 1.763 | 5.2% |
| Sample 3 | 90 | 32.1 | 0.430 | 1.3% | 0.442 | 1.4% | 0.591 | 1.8% | 1.971 | 6.1% | 2.048 | 6.4% |
| Sample 4 | 90 | 515 | 13.244 | 2.6% | 15.960 | 3.1% | 20.022 | 3.9% | 30.946 | 6.0% | 36.160 | 7.0% |
| Sample 5 | 90 | 1209 | 55.556 | 4.6% | 35.986 | 3.0% | 62.186 | 5.1% | 46.448 | 3.8% | 75.930 | 6.3% |
| Sample 6 | 90 | 1402 | 76.545 | 5.5% | 65.882 | 4.7% | 96.037 | 6.8% | 55.619 | 4.0% | 106.997 | 7.6% |

* BAU/mL values for the Negative Controls fall below the assay range and were evaluated based on RLUs instead of BAU/mL.

The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

**14.3 Limit of Blank (LoB)***

Following the method from CLSI EP17-A2, the limit of blank for the LIAISON® SARS-CoV-2 TrimericS IgG assay for serum is 1.55 BAU/mL.

*Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity”.

**14.4 Limit of Detection (LoD)**

Following the method from CLSI EP17-A2, the limit of detection for the LIAISON® SARS-CoV-2 TrimericS IgG assay for serum is 1.85 BAU/mL.

**14.5 Limit of Quantitation (LoQ)**

Following the method from CLSI EP17-A2, the limit of quantitation for LIAISON® SARS-CoV-2 TrimericS IgG assay for serum is 4.24 BAU/mL.

**14.6 Linearity Study**

Three natural infection serum specimens containing high levels of SARS-CoV-2 IgG above the measuring range of the assay at 2080 BAU/mL were diluted with a negative serum to prepare a dilution series comprised of 8 levels spanning approximately 10-20% wider than the assay measuring range. Each level was tested by the LIAISON® SARS-CoV-2 TrimericS IgG assay following CLSI EP6-A. Linearity was demonstrated for the analytical measuring range interval of the assay with deviations from linearity within 15%.

### 14.7 Specimen Equivalency

Matched sample sets from the same donors were used for the matrix comparison studies. Samples contained SARS-CoV-2 IgG levels distributed across the measuring range of the assay. Specimen equivalency was determined by testing the samples with the LIAISON® SARS-CoV-2 TrimericS IgG assay. Results from plasma samples were compared to serum results using a Passing Bablok regression. The resulting equations for each sample type are:

| Sample Type | Slope<br>95% CI | Intercept<br>95% CI | R |
|---|---|---|---|
| Serum SST | 1.01<br>0.99 to 1.03 | -0.05<br>-0.19 to 0.03 | 0.998 |
| EDTA Plasma | 1.06<br>1.04 to 1.08 | -0.24<br>-0.47 to -0.03 | 0.995 |
| Lithium Heparin Plasma | 1.04<br>1.03 to 1.05 | -0.13<br>-0.36 to 0.01 | 0.997 |

*Agreement of the specimen types may vary depending on the study design and sample population used. Assay results obtained at individual laboratories may vary from the data presented.*

## 15. SUMMARY OF CLINICAL PERFORMANCE

### Clinical Agreement

Diagnostic sensitivity and specificity were determined in accordance with CLSI Document EP12-A2.

### 15.1 Clinical Sensitivity

Clinical sensitivity was determined by testing 203 samples collected over the course of time from subjects with a clinical diagnosis of COVID-19 based on a positive SARS-CoV-2 polymerase chain reaction (PCR) method. Twenty-four samples with discordant results to PCR were excluded from the calculation based on their concordance with a comparator IgG serology assay. The following table describes positive percent agreement (PPA) by time of sampling following a positive PCR result.

| Days Post RT-PCR | N | Positive | Negative | PPA | 95% CI (Wilson Score) |
|---|---|---|---|---|---|
| 0-7 | 24 | 16 | 8 | 66.7% | 46.7%-82.0% |
| 8-14 | 24 | 22 | 2 | 91.7% | 74.2%-97.7% |
| ≥15 | 155 | 153 | 2 | 98.7% | 94.5%-99.6% |

### 15.2 Clinical Specificity

Clinical specificity was evaluated by testing 1899 presumed SARS-CoV-2 negative samples from US blood donors collected prior to the COVID-19 outbreak. The following table describes negative percent agreement (NPA).

| Population | N | Positive | Negative | NPA | 95% CI (Wilson Score) |
|---|---|---|---|---|---|
| Apparently Healthy | 1899 | 10 | 1889 | 99.5% | 99.0%-99.7% |

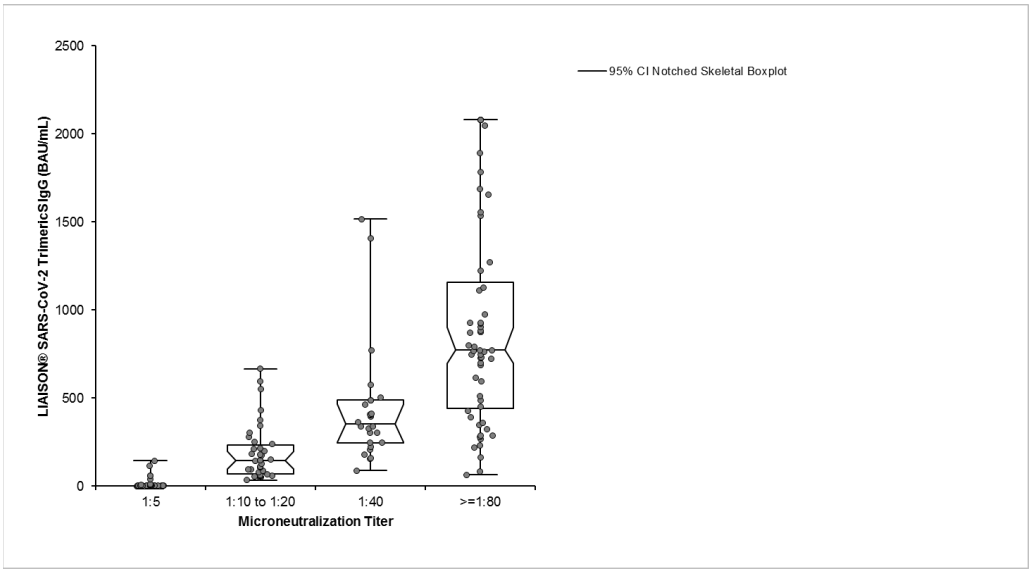
### 15.3 Concordance with Microneutralization Assay

Concordance with neutralizing antibody titers was evaluated by testing 282 samples with Microneutralization assay results. The following table describes negative and positive agreement to 160 Microneutralization assay negative and 122 Microneutralization assay positive (i.e. titer ≥1:10) specimens, respectively.

| LIAISON® SARS-CoV-2 TrimericS IgG | Microneutralization assay Titer | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Negative (< 33.8 BAU/mL) | 155 | 0 | 155 |
| Positive (≥ 33.8 BAU/mL) | 5 | 122 | 127 |
| Total | 160 | 122 | 282 |

| | Proportion | | Wilson 95% CI |
|---|---|---|---|
| Negative Agreement | 96.9% | (155/160) | 92.9% - 98.7% |
| Positive Agreement | 100.0% | (122/122) | 97.8% - 100.0% |

The distribution of LIAISON® SARS-CoV-2 TrimericS IgG assay results by microneutralization titer is shown in the graph below.




Results of 47 samples with high LIAISON® doses (i.e. ≥ 520 BAU/mL) were compared to a higher microneutralization assay titer threshold of ≥ 1:80 to demonstrate concordance at high neutralizing antibody titers.

| LIAISON® SARS-CoV-2 TrimericS IgG | Microneutralization Assay Titer | | Total |
|---|---|---|---|
| | <1:80 | ≥1:80 | |
| ≥ 520 BAU/mL | 15% (7) | 85% (40) | 47 |

### 15.4 Variant Strain Detection

A panel of 22 serum/plasma specimens from UK, collected from 19 patients diagnosed for COVID-19 by RT-PCR, and infected by virus variants (as demonstrated by sequencing) was tested with the LIAISON® SARS-CoV-2 TrimericS IgG assay to assess the performance of the assay. Serum/plasma specimens were collected between 15 and 34 days from the date of onset of symptoms. All 22 specimens were successfully detected with LIAISON® SARS-CoV-2 TrimericS IgG assay.

| Lineage | Number of specimens | Positive result by LIAISON® SARS-CoV-2 TrimericS IgG | Diagnostic Sensitivity and Wilson 95% CI |
|---|---|---|---|
| B.1.1.7 | 22 | 22 | 100% (95% CI: 85.1 – 100%) |

### 15.5 Immune Response Study in Vaccine Recipients

A prospective study was conducted to evaluate a total of 102 sets of samples collected from apparently healthy adults who were included in the Italian vaccine program, were SARS-CoV-2 IgG negative at the time of vaccine, and have received the full course of Pfizer vaccine, i.e. 2 injections. Pre- and post-vaccination samples were collected over the time as follows, time0 at first vaccine dose (baseline), time1 after 21 days from the first vaccine dose and time2 after 21 days from second vaccine dose

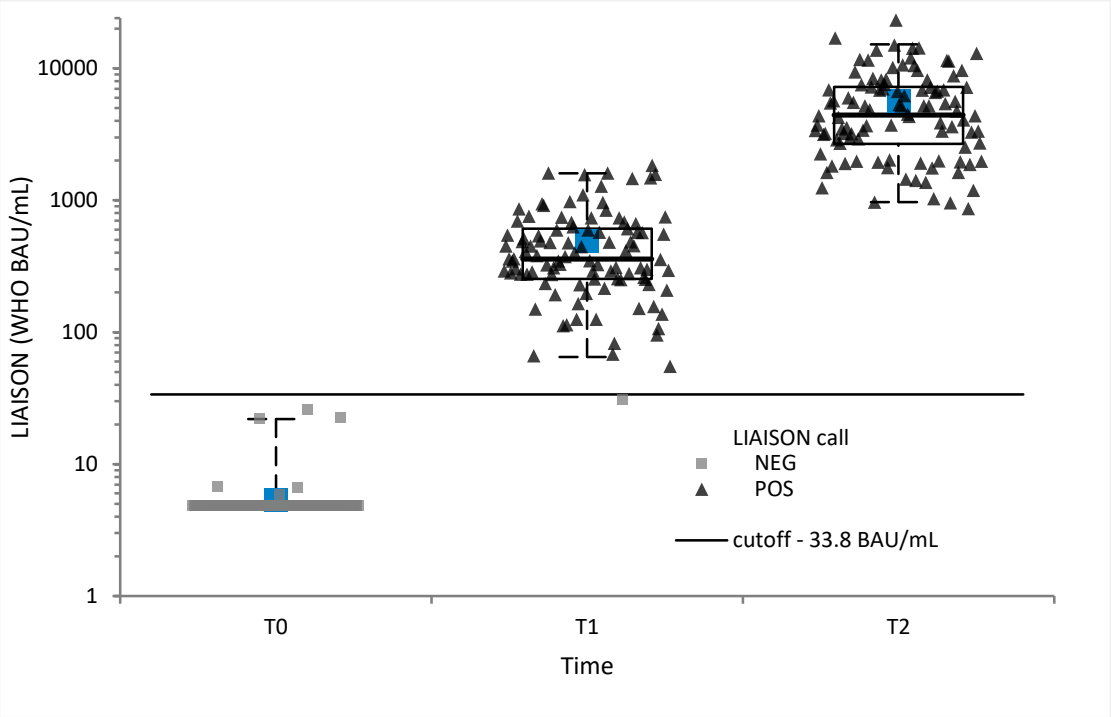
A total of 306 serum samples from 102 subjects were tested with the LIAISON SARS-CoV-2 TrimericS IgG, the results are shown in the table below.

The assay is used to assess the presence of an immune response in vaccine recipients, however the immunity threshold (immune cutoff) that protects from SARS-CoV-2 infection has not been established.

| | Time 0 at first dose | Time 1 at 21 days after first dose | Time 2 at 21 days after second dose |
|---|---|---|---|
| Negative | 102 | 1 | 0 |
| Positive | 0 | 101 | 102 |
| Sensitivity | - | 99.0% | 100% |
| Wilson 95% CI | - | 94.7% - 99.8% | 96.3% - 100% |

Antibodies levels were analysed on vaccinees cohort (n=102) at each time point and the quantitative results expressed as BAU/mL are shown in the table.

| | Time0<br>at first dose | Time 1<br>at 21 days<br>after first dose | Time 2<br>at 21 days<br>after second dose |
|---|---|---|---|
| Number of subjects | 102 | 102 | 102 |
| Median (BAU/mL) | <4.8 | 358.8 | 4435.6 |
| 2.5$^{th}$ percentile (BAU/mL) | <4.8 | 64.9 | 966.1 |
| 97.5$^{th}$ percentile (BAU/mL) | 21.0 | 1604.8 | 15,236.4 |




**16.0 References**


1. European Centre for Disease Prevention and Control (ECDC). Novel Coronavirus. Available from: https://www.ecdc.europa.eu/en/novel-coronavirus-china (last page update March 24 2020)
2. European Centre for Disease Prevention and Control (ECDC). Geographical distribution 2019.
3. Wang G, Jin X. The progress of 2019 Novel Coronavirus (2019-nCoV) event in China. J MedVirol. doi: 10.1002/jmv.25705
4. Kelvin Kai-Wang To, Owen Tak-Yin Tsang et al.: Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. March 23, 2020 Lancet Infectious Diseases
5. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Walls, Park, Tortorici, Wall, McGuire, Veesler. Cell 180, 1–12, March 19, 2020 ª 2020 Elsevier Inc.
6. First WHO International Standard for anti-SARS-CoV-2 Immunoglobulin (Human). NIBSC code: 20/136. Instructions for Use (Version 2.0, Dated 17/12/2020).



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