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CE

LIAISON® SARS-CoV-2 Ag (REF 311490)

1. INTENDED USE

The LIAISON® SARS-CoV-2 Ag assay uses chemiluminescence immunoassay (CLIA) technology for the quantitative determination of SARS-CoV-2 nucleocapsid protein antigen in nasal swab (NS) and nasopharyngeal swab (NPS), in individuals suspected to have COVID-19 by their healthcare provider within the first ten days from the onset of symptoms.

The test has to be performed on the LIAISON® XL Analyzer only.

2. SUMMARY AND EXPLANATION OF THE TEST

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus.

The causative virus of the COVID-19 is called 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.

The incubation period for COVID-19 is thought to range from 2-14 days following exposure, with most cases showing symptoms approximately 4-5 days after exposure[1].

This test identifies the presence of the SARS-CoV-2 in the specimen through the detection of nucleocapsid protein antigen. This antigen is generally detectable in upper respiratory specimens during the acute phase of infection.

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of SARS-CoV-2 Ag in specimens collected and processed through the indicated pre-analytical procedure, is a direct two-step sandwich chemiluminescence immunoassay (CLIA). Specific rabbit polyclonal antibodies to nucleocapsid antigen are used for coating magnetic particles (solid phase) and linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, SARS-CoV-2 viral antigen present in calibrators, samples or controls binds to the conjugate. During the second incubation, the solid phase reacts with the SARS-CoV-2 viral antigen already bound to the conjugate. After the second incubation, the unbound material is 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 in relative light units (RLU) and is directly proportional to the SARS-CoV-2 viral antigen concentration present in calibrators, samples, or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles coated with rabbit polyclonal to SARS-CoV-2 nucleocapsid antigen, BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (1.8 mL)	CAL 1	Antiseptic agent, Recombinant nucleoprotein (from *E.coli*), BSA, detergents. The calibrator concentrations ($TCID_{50}$/mL) are referenced to an inactivated SARS-CoV-2 preparation (bei resources NR52287).
Calibrator 2 (1.8 mL)	CAL 2	Antiseptic agent, Recombinant nucleoprotein (from *E.coli*), BSA, detergents, an inert blue dye. The calibrator concentrations ($TCID_{50}$/mL) are referenced to an inactivated SARS-CoV-2 preparation (bei resources NR52287).
Specimen Diluent (19 mL)	DIL SPE	BSA, phosphate buffer, detergents, ProClin® 300, preservatives, an inert yellow dye.
Conjugate (13 mL)	CONJ	Rabbit polyclonal to SARS-CoV-2 nucleocapsid antigen conjugated to an isoluminol derivative, human serum, BSA, phosphate buffer, detergents, ProClin® 300, preservatives.
Number of tests		100

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

Materials required but not provided

LIAISON® XL Analyzer
LIAISON® XL Cuvettes (REF X0016). LIAISON® XL Disposable Tips (REF X0015) or LIAISON® Disposable Tips (REF X0055). LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300). LIAISON® Wash/System Liquid (REF 319100). LIAISON® XL Waste Bags (REF X0025).

Additional required materials:

LIAISON® Control SARS-CoV-2 Ag (REF 311491).
LIAISON® SARS-CoV-2 Sample Inactivation Buffer (REF 311492).

Recommended materials not supplied in the kit:

Regular polyester swab w/ plastic applicator for nasal specimens, e.g. COPAN Diagnostics

Regular flocked swab w/ plastic applicator for nasal specimens, e.g. COPAN Diagnostics

Minitip or flexible minitip w/plastic applicator for nasopharyngeal specimens transported in viral transport media (VTM) or Universal Transport Media (UTM, COPAN Diagnostics).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

All human serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2, and found to be non-reactive. However, as no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

Visually inspect the integral vials for leaks at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics during pre-analytical procedures and testing activities.

Do not pipette by mouth.

Wear suitable protective clothing, gloves, eye/face protection when handling samples.

Use appropriate precautions in the collection, handling, and storage of patient samples. Refer to the CDC Interim Guidelines for Collection, Handling and Transportation of clinical specimens from persons with Coronavirus Disease 2019 (COVID-19), and to the WHO's Interim guidance for Laboratory testing for coronavirus disease (COVID-19) in suspected human cases at http://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117, as amended and supplemented. Refer to the WHO website for additional publications.

Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol as droplets are a means of transmission of SARS-CoV-2 virus.

All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infectious waste.

Used swabs must be treated as infectious waste.

All samples, **even after the inactivation procedure**, and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents; accordingly samples, reagents and the waste must be handled with utmost care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

For sample handling please refer to Section 9 - Specimen Collection and Preparation.

Pursuant to EC Regulation 1272/2008 (CLP), hazardous reagents are classified and labeled as follows:

REAGENTS:	DIL SPE, CONJ
CLASSIFICATION:	Skin sens. 1 H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin® 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210, safety data sheets available on request. For additional information see Safety Data Sheets available on www.diasorin.com

7. REAGENT PREPARATION

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

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 on 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. Carefully wipe the surface of each septum to remove any residual liquid.

Repeat as necessary until the magnetic particles are completely resuspended.

An incomplete magnetic particle resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

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

Visually inspect the reagents, in particular the calibrators (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foam is present after the resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. Load the integral into the reagent area once the foam has dissipated.

Loading the integral into the reagent area

- LIAISON® XL Analyzer is equipped with a built-in solid-state magnetic device which aids the dispersal of microparticles prior to placing 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 use. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

8. REAGENT INTEGRAL STORAGE AND STABILITY

- **Sealed**: Stable at 2-8°C until the expiry date.
- **Opened on board or at 2-8°C**: stability up to 1 week.
- Use the storage rack provided with the analyzer for the upright storage of the reagent integral.
- Do not freeze.
- Keep upright for storage to facilitate later proper resuspension of the magnetic particles.
- Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

Acceptable specimen types include:

– Dry Nasal swab (NS) processed following the below indicated pre-analytical procedure.
– Nasopharyngeal Swab (NPS) transported in Universal Transport Media (UTM, Copan) or Viral Transport media, then processed following the pre-analytical procedure indicated below.

WARNING: for the collection and handling of swab specimens from the upper and lower respiratory tract, refer to the CDC Interim Guidelines for Collection, Handling and Transportation of clinical specimens from persons with Coronavirus Disease 2019 (COVID-19), and to the WHO's Interim guidance for Laboratory testing for coronavirus disease (COVID-19) in suspected human cases at http://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117, as amended and supplemented. Refer to the WHO website for additional publications.

PRE-ANALYTICAL PROCEDURE WORKFLOW FOR DRY SWAB

1. Collect the sample by nasal swab and place it dry into its container.
2. Dry swab placed into the container can be stored for up to 6 hours at 2°-8°C, prior to transferring the sample into the inactivation buffer.
3. All the following pre-analytical steps should be performed at room temperature (15°-25°C) using the universal precautions for handling potentially infectious specimens.
4. All clinical samples and reagents must be at room temperature (15°-25°C) before beginning the next step of the procedure.
5. Place and soak the swab in the pre-filled tube containing the Sample Inactivation Buffer (REF 311492).
6. Roll the swab at least 5 times while pressing the head against the bottom and side of the tube.
7. Leave the swab in the tube with the inactivation buffer for at least 1 minute.
8. Roll the head of the swab at least 5 times against the inside wall of the tube and remove it.
9. Dispose of the used swab in the biohazardous waste collection.
10. Cap the tube and incubate for at least 120 minutes before handling it.
    Warning: Ensuring that the incubation time lasts at least 120 minutes is essential to properly manage the virus inactivation process and reduce the risk of potential infection. The Laboratory is responsible for the proper recording of this inactivation timing for each of the specimens.Samples that have undergone the pre-analytical inactivation process should be handled and disposed of as though they were potentially infectious.
11. Remove the cap and place the tube on board the instrument for testing. Samples should be tested as soon as possible after the completion of the pre-analytical procedure. If immediate testing is not possible, inactivated samples can be stored at 2°-8°C for up to 6 days or at -20°C or colder for up to 1 month prior to testing. If samples are stored frozen, mix thawed samples well before testing. Frozen samples can undergo up to three freeze/thaw cycles without experiencing any change in performance. Samples after thawing may require centrifugation (i.e. 3,000g x 10') before testing.

PRE-ANALYTICAL PROCEDURE WORKFLOW FOR SWAB IN UNIVERSAL TRANSPORT MEDIUM (UTM) OR VIRAL TRANSPORT MEDIUM (VTM)

1. Collect the sample by nasopharyngeal swab (not provided in the kit) transported in VTM/UTM.
2. Nasopharyngeal swab in VTM/UTM can be stored for up to 12 hours at 2°-8°C, prior to transferring the sample into the inactivation buffer
3. All clinical samples and reagents must be at room temperature (15°-25°C) before beginning the next step of the procedure.
4. All the following pre-analytical steps should be performed at room temperature (15°-25°C) using the universal precautions for handling potentially infectious specimens.
5. Add 1 mL of the specimen eluted in UTM/VTM into the tube containing the Sample Inactivation Buffer (REF 311492).
6. Cap the tube and mix the specimen by vortex for 5-10 sec.
7. Incubate the tube at RT for at least 120 minutes before handling it.
    Warning: Ensuring that the incubation time lasts at least 120 minutes is essential to properly manage the virus inactivation process and reduce the risk of potential infection. The Laboratory is responsible for the proper recording of this inactivation timing for each of the specimens. Samples that have undergone the pre-analytical inactivation process should be handled and disposed of as though they were potentially infectious.
8. Remove the cap and place the tube on board the instrument for testing. Samples should be tested as soon as possible after the completion of the pre-analytical procedure. If immediate testing is not possible, inactivated samples can be stored at 2°-8°C for up to 5 days or at -20°C or colder for up to 1 month prior to testing. If samples are stored frozen, mix thawed samples well before testing. Frozen samples can undergo up to one freeze/thaw cycle without experiencing any change in performance. Freshly collected specimens or samples after thawing may require centrifugation (i.e. 3,000g x 10') before testing.

Inadequate sample collection, handling, storage, or transport may yield erroneous results.

For both procedures, the minimum volume required for a single determination is 400 µL of specimen (100 µL specimen + 300 µL dead volume).

10. CALIBRATION

By testing the assay specific calibrator, the detected relative light unit (RLU) values can adjust the assigned master curve.

Each calibration solution is sufficient for performing 4 calibrations.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

– A new lot of Starter Kit is used.
– The previous calibration was performed more than 1 week before.
– Each time a new lot of integral is used.
– The analyzer has been serviced.
– Control values lie outside the expected ranges.

LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.

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 instructions.

The analyzer operations are as follows:

1. Dispense the specimens (calibrator or control) and conjugate into the reaction cuvettes.
2. Incubate
3. Dispense the Specimen Diluent and magnetic particles into the reaction cuvettes.
4. Incubate and wash
5. Add the Starter Reagents and measure the light emitted.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® SARS-CoV-2 Ag controls:
(a) at least once per day of use, before running the test,
(b) whenever the kit is calibrated,
(c) whenever a new lot of Starter Reagents is used, or in agreement with the guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated, and the controls retested. If the control values obtained after successful calibration repeatedly lie outside the predefined ranges, the test should be repeated using an unopened control vial. If the control values lie outside the expected ranges, patient results must not be reported.

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

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates SARS-CoV-2 Ag concentrations expressed as $TCID_{50}$/mL and grades the results.

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

Assay range: The analyzer directly calculates SARS-CoV-2 viral concentration up to 10^5 $TCID_{50}$/mL.

Samples containing antigen levels above the assay range may be prediluted by the Dilute function of the instrument and retested (the recommended dilution factor is 1:10). The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integrals allows up to 10 samples predilutions to be performed.

Sample results should be interpreted as follows:

LIAISON® SARS-CoV-2 Ag assay		
$TCID_{50}$/mL	**Result**	**Rules and interpretation**
< 200.00	Negative	A result below 200 $TCID_{50}$/mL may indicate the absence of SARS-CoV-2 antigen in the specimen.
≥ 200.00	Positive	A result above or equal to 200 $TCID_{50}$/mL generally indicates presence of the SARS-CoV-2 antigen in the specimen.

A Negative result does not rule out infection by SARS-CoV-2. Negative results should be considered in the context of patient's history, the presence of clinical signs and symptoms consistent with COVID-19, other diagnostic procedures available to the physician, and potential recent exposures to infected subjects. Despite a Negative result, confirmation with molecular assay may be performed, if necessary for patient management.

A Negative result may occur if the sample was collected, stored, or transported improperly.

A Positive result indicates the presence of viral antigens, but clinical correlation with the patient's history and other diagnostic information is necessary to determine the infection status.

A Positive result does not rule out bacterial infection or co-infection with other viruses.

The agent detected may not be the definite cause of disease.

A failure to follow the indicated pre-analytical procedures may adversely affect the test performance.

14. LIMITATIONS

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

Bacterial contamination or heat inactivation of the specimens may affect the test results.

Performance has not been established for use with specimens other than those collected in the upper and lower respiratory tract in humans.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. 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, haemolysis, effects of sample treatment), or cross-reactants.

Endogenous and Exogenous substances Interference

A study was performed to demonstrate that potentially endogenous and exogenous interfering substances potentially found in the upper respiratory tract do not cross-react or interfere in the LIAISON® SARS-CoV-2 Ag assay, at the indicated concentrations.

Substance	Concentration
Whole Blood (Hemoglobin)	50 mg/dL
Menthol	1.5 mg/mL
Diclonine + Menthol	1.5 mg/mL
Benzocain + Menthol	1.5 mg/mL
Phenylephrine	15% v/v
Oxymetazoline nasal spray	15% v/v
Cromolyn nasal spray	15% v/v
Fluticasone propionate	0.125 mg/dL
Homeopathic nasal spray	5% v/v
Homeopathic isotonic nasal spray	1:10 dilution
Fisherman's Friend®	1.5 mg/mL
Tobramycin	4 µg/mL
Oseltamivir phosphate	5 mg/mL

Cross-reactivity and Interference by microorganisms and viruses

A cross-reactivity and potential interference study for the LIAISON® SARS-CoV-2 Ag assay was evaluated by testing various microorganisms and viruses with the LIAISON® SARS-CoV-2 Ag assay. Each organism and virus was tested in triplicate in the absence or presence of 10^3 $TCID_{50}$/mL of inactivated SARS-CoV-2. The tested concentration of the microorganisms and viruses are documented in the Table below.

Virus / Microrganism	Concentration tested	Cross-reactive result (Contains Virus/Micro-organism under evaluation)	Interference result (Contains Virus/Micro-Organism under evaluation and inactivated SARS-CoV-2)
Adenovirus	10^5 PFU/mL	Negative	Positive
Coronavirus 229E	10^5 PFU/mL	Negative	Positive
Coronavirus NL63	10^4 PFU/mL	Negative	Positive
Coronavirus OC43	10^4 PFU/mL	Negative	Positive
Enterovirus 68	10^5 PFU/mL	Negative	Positive
Human Metapneumovirus (hMPV)	10^5 PFU/mL	Negative	Positive
Influenza A H1 N1	10^5 PFU/mL	Negative	Positive
Influenza A H3 N2	10^5 PFU/mL	Negative	Positive
Influenza B	10^4 PFU/mL	Negative	Positive
MERS Coronavirus	0,0595 mg/mL	Positive	Positive
Parainfluenza Virus Type 1	10^5 PFU/mL	Negative	Positive
Parainfluenza Virus Type 2	10^5 PFU/mL	Negative	Positive
Parainfluenza Virus Type 3	10^5 PFU/mL	Negative	Positive
Parainfluenza Virus Type 4b	10^4 PFU/mL	Negative	Positive
Respiratory Syncytial Virus	10^5 PFU/mL	Negative	Positive
Rhinovirus	10^5 PFU/mL	Negative	Positive
SARS Coronavirus	N/A**	Negative	Positive
Bordetella pertussis	10^6 CFU/mL	Negative	Positive
Candida albicans	10^6 CFU/mL	Negative	Positive
Chlamydia pneumoniae	10^6 CFU/mL	Negative	Positive
Haemophilus influenzae	N/A**	Negative	Positive
Legionella pneumophila	10^6 CFU/mL	Negative	Positive
Mycobacterium tuberculosis	10^4 CFU/mL	Negative	Positive
Mycoplasma pneumoniae	10^5 CFU/mL	Negative	Positive
Pneumocystis Carinii	5x 10^6 nuclei/mL	Negative	Positive
Streptococcus pneumoniae	10^4 CFU/mL	Negative	Positive
Streptococcus pyogenes	10^4 CFU/mL	Negative	Positive

*Coronavirus HKU1 was not tested for cross-reactivity due to lack of virus availability.

** Material was not quantified, therefore no tested concentration can be reported. The material was tested at a dilution of 1:20.

Based on the data generated during this study, only MERS Coronavirus demonstrated evidence of cross-reactivity with the LIAISON® SARS-CoV-2 Ag assay.

15.2. Precision

A five-day precision study was performed using a coded panel of 6 contrived samples to obtain samples across the assay range. Kit Controls were also included in the study. The panel of samples and kit controls were tested with the LIAISON® SARS-CoV-2 Ag assay in 6 replicates per run, 3 runs per day for 5 operating days on 2 kit lots, at 2 different sites, on 1 LIAISON® XL Analyzer at each site. The CLSI document EP5-A3 was consulted when preparing the testing protocol.

LIAISON® SARS-CoV-2 Ag Assay, Lot 1, Site 1										
ID	N	Mean ($TCID_{50}$/mL)	Within run		Between run		Between day		Overall	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
Kit Neg	90	1435.5*	41.5	2.9%	29.444	2.1%	49.712	3.5%	71.114	5.0%
Kit Pos	90	856.33	13.1	1.5%	12.341	1.4%	29.114	3.4%	34.225	4.0%
COVAG-01-U1	90	146.37	4.1	2.8%	1.307	0.9%	14.134	9.7%	14.788	10.1%
COVAG-01-U2	90	309.26	5.9	1.9%	2.07	0.7%	17.603	5.7%	18.695	6.0%
COVAG-01-U3	90	461.12	8.7	1.9%	6.443	1.4%	24.367	5.3%	26.677	5.8%
COVAG-01-U4	90	1108.9	18.7	1.7%	8.572	0.8%	24.278	2.2%	31.828	2.9%
COVAG-01-U5	90	8731.6	185.3	2.1%	531.327	6.1%	0**	0.0%	562.704	6.4%
COVAG-01-U6	90	70693	1478.4	2.1%	933.583	1.3%	1948.331	2.8%	2617.856	3.7%

* Elaboration performed on RLU values.

LIAISON® SARS-CoV-2 Ag Assay, Lot 2, Site 2										
ID	N	Mean ($TCID_{50}$/mL)	Within run		Between run		Between day		Overall	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
Kit Neg	90	1398.4*	38.0	2.7%	12.781	0.9%	32.698	2.3%	51.756	3.7%
Kit Pos	90	989.71	15.0	1.5%	0	0.0%	26.854	2.7%	30.749	3.1%
COVAG-01-U1	90	174.88	4.5	2.6%	13.258	7.6%	3.537	2.0%	14.454	8.3%
COVAG-01-U2	90	355.86	4.9	1.4%	17.925	5.0%	16.747	4.7%	25.015	7.0%
COVAG-01-U3	90	515.49	8.7	1.7%	56.407	10.9%	53.339	10.3%	78.123	15.2%
COVAG-01-U4	90	1285	8.7	0.7%	56.407	4.4%	53.339	4.2%	78.123	6.1%
COVAG-01-U5	90	8076	157.1	1.9%	644.529	8.0%	1331.778	16.5%	1487.866	18.4%
COVAG-01-U6	90	61348	1146.9	1.9%	562.09	0.9%	4713.655	7.7%	4883.626	8.0%

* Elaboration performed on RLU values.

LIAISON® SARS-CoV-2 Ag Assay, Two Lots Combined1												
	N	Mean ($TCID_{50}$/mL)	Within run		Between run		Between day		Between-Lot		Overall	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Kit Neg	180	1417*	39.8	2.8%	22.7	1.6%	42.1	3.0%	16.7	1.2%	64.4	10.7%
Kit Pos	180	923.02	14.1	1.5%	8.4	0.9%	28.0	3.0%	93.4	10.1%	98.9	10.7%
COVAG-01-U1	180	160.63	4.3	2.7%	9.4	5.9%	10.3	6.4%	19.5	12.1%	24.4	15.2%
COVAG-01-U2	180	332.56	5.4	1.6%	12.8	3.8%	17.2	5.2%	31.9	9.6%	38.8	11.7%
COVAG-01-U3	180	488.31	8.7	1.8%	40.1	8.2%	41.5	8.5%	32.0	6.6%	66.6	13.6%
COVAG-01-U4	180	1197	20.7	1.7%	17.0	1.4%	15.8	1.3%	124.3	10.4%	128.1	10.7%
COVAG-01-U5	180	8404	171.8	2.0%	590.6	7.0%	938.5	11.2%	122.3	1.5%	1128.7	13.4%
COVAG-01-U6	180	66020	1323.1	2.0%	770.6	1.2%	3606,6	5.5%	6403.1	9.7%	7506.8	11.4%

* Elaboration performed on RLU values.

These precision performance data refer to the repeatability and reproducibility of the analytical assay, not considering the variability of the sample collection and of the pre-analytical procedure of the sample.

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

15.3. Linearity

Linearity was evaluated according to CLSI EP6-A. One sample was tested neat and after dilutions with the Sample Inactivation Buffer. The results were analyzed as a linear regression of the Expected vs. Observed values.

The resulting regression equation is: Observed = 0.956 (Expected) + 247.76; R^2= 0.999

15.4. Analytical sensitivity (LoD)

Analytical sensitivity as LoD was determined for each sample matrix individually, testing samples without analyte and samples with a low level of analyte using two (2) reagent lots on one (1) instrument, in two (2) replicates over three (3) days, with the following results: LOD = 22.0 $TCID_{50}$/mL

15.5. Hook effect

The highest concentration of inactivated SARS-CoV-2 stock available (10^6 $TCID_{50}$/mL) was tested and no hook effect was observed.

16. SUMMARY OF CLINICAL PERFORMANCE

Nasal swabs

The clinical performance of the LIAISON® SARS-CoV-2 Ag test was established with a total of 147 nasal swab samples collected from individual subjects during the 2020 COVID-19 pandemic. Specimens were collected from 2 different vendors (in the US) and 1 clinical center (in Europe) from symptomatic patients suspected of COVID19. Nasal swabs were collected and eluted in Sample Inactivation Buffer (REF 311492) and stored frozen until tested. Samples were thawed and tested with LIAISON SARS-CoV-2 Ag accordingly to the assay procedure. All subjects were confirmed as positive (≤10 days from onset of symptoms) or negative by a reference extracted RT-PCR method, used as comparator method for the study.

		Reference extracted RT-PCR assay		
		POS	**NEG**	**Total**
LIAISON® SARS-CoV-2 Ag on nasal swabs	**POS**	34	0	34
	NEG	1	112	113
	Total	35	112	147

Positive Percentage Agreement (Sensitivity): 34/35 (97.1%, 95% CI: 85.5 – 99.5%).
Negative Percentage Agreement (Specificity): 112/112 (100%, 95% CI: 96.7 – 100%).

Nasopharyngeal swabs

The clinical performance of the LIAISON® SARS-CoV-2 Ag test was established with a total of 240 nasopharyngeal swab collected from individual subjects during the 2020 COVID-19 pandemic. Specimens were collected from 2 different vendors (1 in the US, 1 in Europe) and 1 clinical center (in Europe) from symptomatic patients suspected of COVID-19. Nasopharyngeal swabs were collected in UTM/VTM, stored frozen, thawed and eluted in Sample Inactivation Buffer (REF 311492), tested with LIAISON® SARS-CoV-2 Ag accordingly to the assay procedure. All subjects were confirmed as positive (≤10 days from onset of symptoms) or negative by a reference extracted RT-PCR method, used as comparator method for the study.

		Reference extracted RT-PCR assay		
		POS	**NEG**	**Total**
LIAISON® SARS-CoV-2 Ag on nasopharyngeal swabs	**POS**	35	1	36
	NEG	2	202	204
	Total	37	203	240

Positive Percentage Agreement (Sensitivity): 35/37 (94.6%, 95% CI: 82.3 – 98.5%).
Negative Percentage Agreement (Specificity): 202/203 (99.5%, 95% CI: 97.3 – 99.9%).

The clinical performance of the LIAISON® SARS-CoV-2 Ag test was evaluated with positive results stratified by the RT-PCR method cycle threshold (Ct) counts. As presented in the table below, the positive agreement of the LIAISON® SARS-CoV-2 Ag test is higher with samples of a Ct count <33.

		Reference extracted RT-PCR assay		
		< 33Ct	**≥ 33Ct**	**Total**
LIAISON® SARS-CoV-2 Ag on nasopharyngeal swabs	**POS**	34	1	35
	NEG	1	1	2
	Total	35	2	37

Positive Percentage Agreement (Sensitivity) for Ct <33: 34/35 (97.1%, 95% CI 85.5 – 99.5%).

LIAISON® Control SARS-CoV-2 Ag (REF 311491)

1. INTENDED USE

The LIAISON® SARS-CoV-2 Ag controls (negative and positive) are intended for use as assayed quality control samples to monitor the performance and reliability of the LIAISON® SARS-CoV-2 Ag assay. The performance characteristics of the LIAISON® SARS-CoV-2 Ag controls have not been established for any assays or instrument platforms other than LIAISON® XL.

The certificate of analysis barcodes give specific information on the lot of controls and should be read by the hand-held barcode scanner of the LIAISON® XL Analyzer prior to loading the control vials on board. For details, refer to the analyzer operator's manual.

2. MATERIALS PROVIDED

Negative control (2 x 2.7 mL)	CONTROL -	Antiseptic agent, BSA, detergents.
Positive control (2 x 2.7 mL)	CONTROL +	Antiseptic agent, recombinant nucleoprotein (from *E.coli*), BSA, detergents.

All reagents are supplied ready to use. The range of values 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. Each laboratory is responsible for adopting different limits to meet individual requirements.

3. WARNINGS AND PRECAUTIONS

– For *in vitro* diagnostic use.
– Controls are not kit lot specific and may be safely interchanged even with different reagent integral lots.
– Observe the normal precautions required for handling all laboratory reagents.
– Dispose of all waste material in accordance with local guidelines.

4. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory. Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infectious waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

5. STORAGE AND STABILITY

Upon receipt, the controls must be stored at 2-8°C in an upright position to prevent adherence of the solution to the vial cap. Do not freeze.

When the controls are stored sealed, and kept upright, they remain stable at 2-8°C until the expiry date.

Once opened, the controls are stable for up to 2 weeks when properly stored at 2-8°C between two successive uses.

Avoid bacterial contamination of the controls.

The controls should not be used past the expiry date indicated on the vial labels.

6. PREPARATION OF REAGENTS

– Place the control vials in type C racks on the analyzer. Each control vial is sufficient for performing at least 20 tests.
– The dead volume is 400 µL.
– At the time of use, equilibrate the controls to room temperature (20-25°C) before opening the vials and keep them on board the instrument only for the amount of time required for quality control testing.
– After use, stopper the vials promptly and store them at 2-8°C in an upright position.
– During handling, use appropriate precautions to avoid bacterial contamination of the controls.

7. LIMITATIONS

Control values for assays other than the LIAISON® SARS-CoV-2 Ag assay have not been established. If users wish to use this control material with other assays, it is their responsibility to establish the appropriate ranges.

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

If the control values obtained after successful calibration repeatedly lie outside the expect ranges, the test should be repeated using an unopened control vial.

8. HANDLING

For proper handling please refer to the analyzer operator's manual.

9. ASSIGNED VALUES

The ranges of SARS-CoV-2 Ag concentration in the controls are printed on the certificate of analysis. They have been established after taking into account run variability, in order to guarantee the accuracy of analytical results and to obtain indications on the stability or deterioration of reagents.

LIAISON® SARS-CoV-2 Sample Inactivation Buffer (REF 311492)

1. INTENDED USE

Sample Inactivation Buffer is used in association with nasal swab (NS) and nasopharyngeal swab (NPS) (dry or eluted in viral transport media as UTM/VTM) and is intended todecrease the viral load of SARS-CoV-2 in the specimen by inactivationduring the pre-analytical procedure, when testing the samples with the LIAISON® SARS-CoV-2 Ag assay.

The performance characteristics of LIAISON® SARS-CoV-2 Sample Inactivation Buffer have not been established for any assays or instrument platforms other than LIAISON® XL.

2. MATERIALS PROVIDED

Sample Inactivation Buffer (100 x 1.0 mL)	BUF	Antiseptic agent, detergents, and inert blue dyes.

Supplied ready to use.

3. WARNINGS AND PRECAUTIONS

– For *in vitro* diagnostic use.
– Sample shall be collected following the manufacturer's instructions for use and the COVID-19 standard of care.
– Observe the normal precautions required for handling all laboratory reagents.
– Dispose of all waste material in accordance with local guidelines.

4. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory. Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infectious waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use reagents beyond the expiration date given on the label.

5. STORAGE AND STABILITY

Keep away from sunlight. 

Upon receipt, the vials must be stored at 2-8°C in an upright position to prevent adherence of the solution to the vial cap.

Do not freeze.

When the vials are stored sealed and kept upright, they remain stable at 2-8°C until the expiry date.

Avoid bacterial contamination.

The reagent should not be used past the expiry date indicated on the vial labels.

6. LIMITATIONS

Do not use to inactivate any viruses other than SARS-CoV-2.

7. HANDLING

For proper handling please refer to the LIAISON® SARS-CoV-2 Ag Instructions for Use.

REFERENCES

1. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020;172(9):577-582. doi:10.7326/M20-0504.

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