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smarter diagnostics

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1 PRODUCT DESCRIPTION

1.1 Name of the product

Certest SARS-CoV-2 (SC) Test.

1.2 Product Technology

Certest SARS-CoV-2 Test is an Immunochromatographic Test for the rapid detection of antigens in respiratory samples (nasopharyngeal).

1.3 Intended Use

Certest SARS-CoV-2 Test has been designed for the rapid detection of *Coronavirus SARS-CoV-2* antigen in human nasopharyngeal swab specimens, by health care professionals, to aid in the diagnosis of Coronavirus (SARS-CoV-2) respiratory infection.

2 INTRODUCTION AND CONTEXT

The object of this document is to define and execute the performance evaluation of the Lateral Flow product indicated in section 1. The document will include all the relevant information related to this evaluation.

This Performance Evaluation is defined in Document ñPOC-51, Evaluación de Funcionamientoò (Performance Evaluation).

3 PLANNING OF THE EVALUATION

The Certest SARS-CoV-2 Test is a new development.

The described information has been collected along the validation of the new test: Certest SARS-CoV-2 Test.

The information is going to be updated every year with historical data regarding the analytical sensitivity of the lots produced during the year.

Any new clinical evaluation (external or internal) will be also added to this document.

4 LOCATION

Most of the evaluation measurements -except any external clinical evaluations- have been performed in our facilities (CerTest Biotec S.L., Polígono Industrial Rio Gállego II, Calle J, Nº1, 50840, San Mateo de Gallego, Zaragoza, Spain).

When the experiments have been performed in other locations, they will be indicated in the corresponding point / section.

5 DATES

The dates of every measurement are indicated in each of the sections.

6 RESPONSIBILITIES

The Design and Final Approval of this full evaluation is responsibility of its coordinator, the Product specialist: Dr. Manuel Villacampa.

The evaluation performed at CerTest Biotec has been made by several CerTest Laboratory Technicians. Names are not detailed in this document (L.O. 3/2018, de 5 diciembre sobre protección de datos personales y Garantía de derechos digitales, LOPD-Gdd).

Any External Measurement will be detailed in the correspondent Section or Document, including researchers, technicians, location and dates.

7 SAMPLES

The Samples that must be employed for running the test are human nasopharyngeal samples. They should be analysed as soon as possible.

The real samples used for this study are in most cases -and when not specify- nasopharyngeal samples from different providers. The samples were most of the times diluted in UTM (Universal Transport Media), VTM (Viral Transport Media) or in a SB (Saline Buffer), for their posterior use in MDx measurement.

For most of the measurements of analytical sensitivity for Certest SARS-CoV-2 Test were employed different antigens, lots and concentrations. The different concentrations were prepared by diluting these antigens into the sample buffer or in a negative human nasopharyngeal sample.

ANTIGENS USED IN THIS DOCUMENT
Coronavirus SARS-CoV-2
Nucleoprotein (RI-4409I) SARS-CoV-2 recombinant Nucleoprotein mamalian HEK293 cells Lot: C186NP- C012; Exp. Date: NA; conc:1.35 mg/mL Lot: C186NP- C002 (AgCV-00002); Exp. Date: 2021-04; conc:1.00 mg/mL Lot: C186NP- C009 (AgV-00003); Exp. Date: 2021-06; conc:0.7 mg/mL
Zeptometrix Culture (RI-4475) SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (0.5 mL) Lot 324609; Exp. Date: 2023-07-08. Lot 324774; Exp. Date: 2023-07-27.

8 DEFINITIONS

Definitions:

Analytical Sensitivity: It is the minimum concentration that a test can detect.

Reproducibility: refers to the variation in measure (measurements) made on a subject under changing conditions. The changing conditions may be due to different measurement methods or instruments being used, measurements being made by different observers or raters, or measurements being made over a period of time, within which the óerror- freeô level of the variable could undergo non-negligible change.

Repeatability: refers to the variation in repeat measurements made on the same subject under identical conditions. This means that measurements are made by the same instrument or method, the same observer (or rater) if human input is required, and that the measurements are made over a short period of time, over which the underlying value can be considered to be constant. Variability in measurements made on the same subject in a repeatability study can then be ascribed only to errors due to the measurement process itself.

Lateral Flow: Diagnostic Detection Immunological Technology based on the capture visible of colloidal conjugates on specific areas of a solid porous support.

Abbreviations:

+: positive sample	**-:** negative sample
±: low positive value	**LF**: Lateral Flow
Min: minute	**CI:** confidence interval

PPV: positive predictive value	**NPV:** negative predictive value
IFU: instructions for use	**not ev.:** not evaluated
RT: Room temperature (15-25ºC)	**NA:** Not applicable
MDx: Molecular immunodiagnostic	**IC-Test**:Immunochromatographic test
RH: Relative humidity	**LOD:** Limit of Detection

9 MATERIALS EMPLOYED

For the evaluation of Certest SARS-CoV-2 Test, the following materials have been selected:

¶ Certest SARS-CoV-2 Test Lot number used:

General lot	SC-001	**Expiry date**	2022-08
General lot	SC-002	**Expiry date**	2022-08
General lot	SC-003	**Expiry date**	2022-08
General lot	SC-011	**Expiry date**	2022-10
General lot	SC-018	**Expiry date**	2022-11
General lot	SC-019	**Expiry date**	2022-11
General lot	SC-020	**Expiry date**	2022-11

10 METHODOLOGY / PROTOCOL

In most of the experiments, the different concentrations of *Coronavirus SARS-CoV-2* antigen have been prepared by spiking *Coronavirus SARS-CoV-2* antigen to different buffer solutions. Most of the measurements have been performed using the sample dilution buffer for this dilution; in some cases, the antigen has been previously diluted in a negative human nasopharyngeal sample and later, following the test protocol, the sample has been mixed with the sample dilution buffer. In any case, the concentration of the antigen indicated refers always to the concentration present in the final dilution, that measured.

When using samples, they were analysed immediately fresh samples or stored at 2ºC-8ºC till tested.

For running the strips, the protocol specified in the IFU has been followed; the measurements have been always registered after 10 minutes.

If other protocols would be followed will be indicated in each experiment.

11 EXPECTED VALUES

For this evaluation, the expected values are:

- Analytical sensitivities close to 1.00 ng/mL of *Coronavirus SARS-CoV-2* nucleoprotein, or $1 \cdot 10^3 TCID_{50}$/mL of 2019 nCoV/USA-WA1/2020.
- Reproducibility in the order of ± one / two 2-fold dilution.
- Repeatability in the order of ± one / two 2-fold dilution.
- Stability in the order of ± one / two 2-fold dilution.
- According to the latest recommendations of the World Health Organization (WHO)
  - Clinical Sensitivity: better than 80%.
  - Clinical Specificity: better than 97%.

12 EQUIPMENTS

For the current evaluation it was not necessary the use of any equipment (visual interpretation), only follow the procedure of instructions for use.

Anyway, we have available two Qiagen ESEquant LR3 Lateral Flow Strip Readers for strip analysis, if required. (Strip reader: SN# 0035, EQ-842)

We have, also, several Optricon Cube Readers available.

The q-PCR measurements were run at an Aria Mx Real-Time PCR instrument, Model G8830-64001 with SN#: MY165005276 (from Ag Plant Technologies / Agilent) (EQ-949).

To control de test reading time, we use a timer: EQ-1476X (VWR EV No 609-0181 NA No 89203-042 17671080).

13 USE OF THE TEST

The test has always employed following the official test Instructions for Use (IFU's)

The versions employed along the time of this evaluation were:

- IU-SC8 v.02.1 and IU-SC8 v.03, instructions for use, instructions for use.

14 SCIENTIFIC VALIDITY

The detection of any virus can be performed by means of several techniques. Among them, the molecular and the immunological ones are nowadays two of the most widely spread. It is clear that the Molecular technologies (specially q-PCR) are really powerful for the virus detection -with the highest sensitivities and specificities-, but it is also clear that the cost (economical and human) of those techniques is very high, requiring specialized teams, machines, laboratories and larger developing times.

The immunological techniques, specially the rapid test based on immunochromatography can conduct to good enough sensitivities specificities when the test is well designed and includes the best antibodies (normally monoclonal ones). It is true that the performance is not as good as the molecular test, but on the other side they have several advantages such as:

- The technicians do not need a high degree of qualification
- The test can be run in few minutes
- There is no need of instruments
- The price per test is lower than the molecular tests.
- They can be run massively practically with no restriction in number, allowing a general screening of population.

In this moment there are some rapid tests in the market for antigen detection of SARS-CoV-2. Most of them are focused on the detection of the SARS2 nucleoprotein. The doctors are employing them for several purposes -mainly for screening- and consider that their performance is good enough for it. According to the latest recommendations of the World Health Organization (WHO)-***Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays*-11th September 2020-** a sensitivity better than 80% will be expected for samples with high viral loads (corresponding to the interval of up to 7 days after the appearance of symptoms or a Ct equal to or less than 28 cycles) and better than 60% for non-selected samples (including the general population without suspected infection). In terms of specificity, better than 97%.

The use of the Rapid Immunochromatographic Test for the detection of *Coronavirus SARS-CoV-2* antigen can be validated from the following scientific papers:

1. Ghaffari, A.; McGill, I. and Ardakani, A. ***Trends in COVID-19 Diagnostic Test Development***. *BioProcess International.* 8(6): 34. Jun. 2020.

ñ*Our findings identify a significant gap in the development of rapid, accurate, and simple point-of-care COVID-19 diagnostic tests.* ***(é)ò***

VIRAL GENOME SEQUENCING:

ñHigh co*sts associated with instruments, reagents and specially trained staff in addition to the complexity of data analysis.* ***(é)ò***

NUCLEID-ACID AMPLIFICATION TESTS:

ñ(é) *current PCR test requires time-consuming sample preparation, sophisticated instruments and highly trained staff.* ***(é)ò***

VIRAL ANTIGEN TESTS:

ñLat*eral flow assays commonly are used to develop low-cost, simple, rapid and portable antigen-based diagnostic tests.* ***(é)ò***

SEROLOGICAL ANTIBODY TESTS

ñAr*e unsuitable for early diagnosis (7-14 days).* ***(é)ò***

2. Palestino, G.; García-Silva, I.; González-Ortega, O. and Rosales-Mendoza, S. ***Can nanotechnology help in the fight against COVID-19?*** *Expert Review of Anti-infective Therapy*. Jun. 2020. https://doi.org/10.1080/14787210.2020.1776115

ñ*The current COVID-19 pandemic caused by the SARS-CoV-2 virus demands the development of strategies not only to detect or inactivate the virus, but to treat it (therapeutically and prophylactically).* ***(é)ò***

ñ(é) *RT-PCR has the disadvantage of requiring sophisticated equipment and a laboratory with biosafety level 2 or above.* ***(é)*** *Moreover, the time required to obtain the results is up to 3 days. This time-consuming method is exceptionally disadvantageous for public health emergencies such as the one we are currently living since the COVID-19 came out. In this scenario, the development of point-of-care (PoC), low-cost, and efficient devices; capable of providing robust, fast, and reliably responses are urgently needed. (* ***é)ò***

ñTechno*logies to detect or inhibit the virus; and also, to prevent the infection are urgently needed.* ***(é)ò***

3. Pérez-García, F.; Pérez-Tanoira, R.; Romanyk, J.; Arroyo, T.; Gómez-Herruz, P. and Cuadros-González, J. *(* ***é)*** ***rapid lateral þow immunoassays is reliable in diagnosing SARS-CoV-2 infection from 14 days after symptom onset: A prospective single-center study.*** *Journal of Clinical Virology*. Vol. 129, 104473. May. 2020. https://doi.org/10.1016/j.jcv.2020.104473

ñ(é) *those time-consuming tests based on ELISA are not as suitable for clinical use as rapid tests and, as a matter of fact, cannot be included in the management algorithms in emergency departments.* ***(é)ò***

ñThe cu*rrent situation of the COVID-19 pandemic requires an urgent and coordinated answer*

to the inherent problems of the PCR-based diagnosis: on the one hand the low capacity to carry out PCR techniques in some laboratories and also the low sensitivity of PCR test in nasopharyngeal samples, specially from the second week of infection. ***(é)ò***

ñ(é) serolog*y for diagnosis of SARSCoV-2 infection: hey also require special equipment, trained personnel and take several hours to perform.* ***(é)ò***

4. Younes, N.; Al-Sadeq, D. W.; AL-Jighefee, H.; Younes, S.; Al-Jamal, O.; Daas, H. I.; Yassine, H. M. and Nasrallah, G. K. ***Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2.*** *Viruses* (*Multidisciplinary Digital Publishing Institute*), 12 (6): 582. May. 2020.
https://doi.org/10.3390/v12060582

ñThe *past three unprecedented outbreaks of emerging human coronavirus infections at the beginning of the 21st century have highlighted the importance of readily available, accurate, and rapid diagnostic technologies to contain emerging and re-emerging pandemics.* ***(é)ò***

ñ(é) *the r****apid identiýcati****on of suspected cases remains a high priority to properly allocate personal protective equipment (PPE) and to prevent nosocomial spread with subsequent community transmission.* ***(é)ò***

ñAltho*ugh rRT-PCR provides a relatively rapid result (average 3****ï****4 h), it is limited by* ***transportation to the laboratory and the requirement to batch samples in a large run (é). Thus,*** *public health sectors are in deep need for fast and reliable tests for SARS-CoV-2 to be able to effectively contain the pandemic. Cost-efective and eficient diagnostic techniques as near to the POC as possible would be a game-changer in the current situation.* ***(é)ò***

ñ(é) *studies have shown that molecular technologies are more accurate than CT scans and serological tests for the d****eýniti****ve diagnosis of COVID-19, as they can target and identify the* ***speciýc antigen of SARS****-CoV-2. Unfortunately, the currently available diagnostic tests are labor-intensive and time-consuming, and a shortage of commercial kits delays diagnosis.* ***(é)ò***

ñAl*though rRT-****PCR offers many beneýts, it has some limitations. Its low stability and long*** *processing time were detrimental to the health care efforts to contain the outbreak. Also, several external factors may affect rRT-PCR testing results accuracy, including sampling operations, specimen source (e.g., upper or lower respiratory tract), sampling timing (before and after symptoms onset), and the performance of detection kits. (****é)ò***

ñ*Moreover, using PCR, codetection with other respiratory viruses is frequently encountered in coronaviruses (****é****). Furthermore, rRT-PCR requires professionally trained staff to operate sophisticated laboratory facilities, which are usually located at a central laboratory (biosafety level 2 or above) and is often time-consuming (****é****). This often leaves a rapidly rising number of potential cases untested.* ***(é)ò***

ñ(é) *serological test have some disadvantages, mainly involving the slow antibody response*

to SARS-CoV-2 virus, as they may not be detectable until three days from symptom onset or at least 7ï10 days after infection. In addition, these tests are not designed to detect individuals in the early stages of COVID-19 infection. ***(é)ò***

ñ*Another challenge of using manual ELISA for SARS-CoV-2 detection is that IgM antibodies are notoriously non-****speciýc, and given the time it takes for the development of speciýc IgG*** *antibodies, serology testing will not likely play an active role in the detection of early cases except for diagnosis/c****onýrming late ca****ses or to determine the immunity of healthcare personnel as the outbreak progresses. Furthermore, manual ELISA kits are subject to many interferences* ***(é)ò***

ñ*Although VNA (Virus neutralization assay) is very sensitive, it is more complex, time-consuming, and requires labor with good technical skills to conduct the assay compared to other* ***serological tests. (é)ò***

ñDue to the high infectious rate of SARS*-CoV-2, it is essential to have accurate and precise diagnostic technologies as soon as possible* ***(é)ò***

5. Pachito, DV.; Bagattini, AM.; Oliveira, Jr. HA.; Medeiros, FC.; Brito, GV.; Matuoka, JY.; Marra, LP.; Parreira, PCL.; Colpani, V.; Falavigna, M.; Stein, C. and Riera, R. ***Testes diagnósticos para Covid-19. Síntese de evidência.*** May.2020. https://oxfordbrazilebm.com/index.php/2020/03/27/testes-diagnosticos-covid-19

ñU*m dos pontos críticos neste cenário é a disponibilização de testes diagnósticos que sejam rápidos e acurados e que possam ser empregados para confirmação de casos suspeitos, rastreio de população de risco e para avaliação epidemiológica em nível populacional.* ***(é)ò***

ñTecnologias empregadas no *próprio local de atendimento, denominadas point of care, oferecem inúmeras vantagens. Estes testes fornecem resultados rápidos, permitindo a tomada de decisão de forma ágil. A maioria dos testes point of care comercializados na atualidade tem como objetivo a detecção de anticorpos, o que levanta preocupação em relação à possibilidade de baixa sensibilidade do teste em fases iniciais de infecção.* ***(é)ò***

6. Kubina, R. and Dziedzic, A ***Molecular and Serological Tests for COVID-19. A Comparative Review of SARS-CoV-2 Coronavirus Laboratory and Point-of-Care Diagnostics.*** *Diagnostics* (*Multidisciplinary Digital Publishing Institute*), 10 (6): 434. Jun. 2020. https:// doi: 10.3390/diagnostics10060434

ñDue to the c*urrent pandemic situation, a development of point-of-care diagnostics (POCD) allows us to substantially accelerate taking clinical decisions and implement strategic planning at the national level of preventative measures.* ***(é)ò***

ñAs a *result of this rapidly progressing COVID-19 pandemic and the limited laboratory-*

based molecular testing capacities, new point-of-care (POC), scalable rapid diagnostic tests have been invented recently as easy-to-use tools to allow COVID-19 diagnostics outside of laboratory settings. What is more, the urgent need to multiply testing for COVID-19 has been clearly identified as an essential element of the anti-coronavirus strategy all over the world.« ´

³$W WKH WLPH RI LQFUHDVHG GHPDQG IRU KRVSLWDO V services urgently need a fast, sensitive, but at the same time inexpensive diagnostic test, in order to rapidly manage patients, regarding admissions to hospitals meant for COVID-19 treatment. Therefore, the role of an approved and reliable diagnostic test in the COVID-19 care pathway is of the utmost importance.« ´

³7KH standard protocol with the application of the RT-qPCR method is demanding and time-consuming. « ´

7. Matheeussen, V.; M. Corman, V.; Donoso Mantke, O.; McCulloch, E.; Lammens, C.; Goossens, H.; Niemeyer, D.; S. Wallace, P.; Klapper, P.; GM. Niesters, H.; Drosten, C. and Leven, M. International external quality assessment for SARSCoV-2 molecular detection and survey on clinical laboratory preparedness during the COVID-19 pandemic, April/May 2020. Eurosurveillance 25(27): 2001223. Jul. 2020. https://doi.org/10.2807/1560-7917.ES.2020.25.27.2001223

³$FFHVV WR reliable diagnostic assays and adequate testing capacity for the causing severe acute respiratory síndrome coronavirus 2 (SARS-CoV-2) is essential for preparedness and response sWUDWHJLHV ZRUOGZLGH « ´

8. J. Mina, M.; Parker, R. and B. Larremore, D. Rethinking Covid-19 Test Sensitivity ² A Strategy for Containment. The New England Journal of Medicine. Sept. 2020.

³« WKH PRUH UHOHYDQW PHDVXUH RI D WHVWLQJ UHJL SUREDELOLW\ WKDW LQIHFWHG in time to be removed out of the population¶UH L DQG SUHYHQW VSUHDG WR RWKHUV « ´

³7UDQVPLVVLRQ appears to occur 2 days after exposure, when the viral load SHDNV « ´

³%\ VHYHUDO FULWHULD WKH EHQFKPDUN polymerase-chain-reaction (PCR) test F fails when used in a surveillance regimen. After collection, PCR samples typically require transport to a centralized lab staffed by experts, which drives up costs, drives down frequency, and FDQ GHOD\ UHVXOWV E\ RQH RU PRUH GD\V « ´

³« GHVSLWH very high analytic sensitivity of the diagnostic tests deployed for surveillance, WRGD\¶V regimens have at best only 10% sensitivity to detect infections and are failing as

&RYLG ILOWHUV « ´

³ « 0RUHRYHU WKH ZHOO-described long tail of RNA positivity after the transmissible stage means that many, if not most, people whose infections are detected during routine surveillance using high-analytic-sensitivity but low-IUHTXHQF\ WHVWV DUH QR ORQJHU LQIH

³)RU DQ HIIHFWLYH Covid filter that will stop this pandemic, we need tests that can enble regimens that will capture most infections while they are still infectious. These tests exist today in the form of rapid lateral-flow antigen tests« (´

³:H EHOLHYH WKDW VXUYHLOODQFH testing regimens that can sever enough transmission chains to reduce community spread should complement, not replace, our current clinical diagnostic tests. « ´

³7KH)'\$¶V ODWH \$XJXVW HPHUJHQF\ XVH DXWKRUL]DWLR
rapid, instrument-free antigen test to receive an \$EUZDV D VWHS LQ WKH ULJKW GL

³7R GHIHDW &RYLG-19, we believe that the FDA, the CDC, the National Institutes of Health, and others must encourage structured evaluations of tests in the con- text of planned testing regimens to identify WKRVH WKDW ZLOO SURYLGH WKH EHVW &RYLG ILO

Based on this principle, different companies were able to design immunological based test for the detection of the specific target. There are several Lateral Flow Immunochromatographic based product available in the market, which again confirms the validity of this technology for this specific purpose (detection of Coronavirus SARS-CoV-2 antigen) as the sensitivity of the technique is high enough. Examples of previous LF/IC test from the competence are:

- STANDARD F COVID-19 Ag FIA (SD BIOSENSOR, Inc; Brazil ANVISA - CE-IVD)
- STANDARD Q COVID-19 Ag Test (SD BIOSENSOR, Inc; Brazil ANVISA - CE-IVD)
- PANBIO™ COVID-19 Ag rapid Test Device (Abbott)
- SARS-CoV-2 Rapid Antigen Test (Roche Diagnostics)
- Amela Covid-19 Antigen test (Amedica SA, Norway NMA - CE-IVD)
- mariPOC SARS-CoV-2 (ArcDia International Ltd)
- COVID-19 Ag Respi-Strip (Coris BioConcept - CE-IVD)
- GenBody COVID-19 Ag (GenBody, Inc. - CE-IVD)
- 2019-nCoV Antigen Rapid Test Cassette (Hunan Lituo Biotechnology Co., Ltd; India CDSCO - CE-IVD)
- PerfectPOC Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit (Jiangsu Bioperfectus Technologies Co. Ltd; CE-IVD)

- P4DETECT COVID-19 Ag (PRIME4DIA Co., Ltd - RUO)
- BIOCREDIT COVID-19 Ag (RapiGEN Inc. - CE-IVD)
- Bioeasy 2019-nCoV Ag Fluorescence Rapid Test Kit (Time-Resolved Fluorescence) (Shenzhen Bioeasy Biotechnology Co., Ltd - CE-IVD)

15 GENERAL PERFORMANCE RESULTS

15.1 Repeatability

This measurement has been performed by diluting Coronavirus SARS-CoV-2 antigen in a sample dilution buffer. The employed strip lots were SC-001, SC-002 and SC-003. (Laboratory Technician: technician 1, date: 03/09/2020)

For the practical realization of the repeatability, each lot of validation three will be repeated 5 times with the same equipment (in this case micropipette EQ-1849X,1850X,1851X) in the same day and performed by the same technician.

Sensitivity curves will be made, in which both the batch of antigen and buffer will be constant throughout the entire assay. Calculating the repeatability limit (r) as:

$$r = \frac{S_r}{\bar{x}} \times 100$$

The results obtained are:

Laboratory technician	Technician 1				
Product lot	SC-001				
Sample diluent buffer lot	DIL 10-354 (202508)				
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012				
Temp. (ºC) /RH (%)	23.5/ 41.1				
Measuring device	EQ-1033				
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	Repeatability (repetitions)				
	1st	2nd	3rd	4th	5th
32.00	+	+	+	+	+
16.00	+	+	+	+	+
8.00	+	+	+	+	+
4.00	+	+	+	+	+
2.00	+	+	+	+	+
1.00	+	+	+	+	+
0.50	+	-	-	-	+
0.25	-	-	-	-	-
0.13	-	-	-	-	-
0.00	-	-	-	-	-

Laboratory technician	Technician 1				
Product lot	SC-002				
Sample diluent buffer lot	DIL10-355 (2025-08)				
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012				
Temp. (ºC) / RH (%)	23.5 / 41.1				
Measuring device	EQ-1033				
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	Repeatability (repetitions)				
	1st	2nd	3rd	4th	5th
32.00	+	+	+	+	+
16.00	+	+	+	+	+
8.00	+	+	+	+	+
4.00	+	+	+	+	+
2.00	+	+	+	+	+
1.00	+	+	+	+	+
0.50	-	±	±	±	±
0.25	-	-	-	-	-
0.13	-	-	-	-	-
0.00	-	-	-	-	-

Laboratory technician	Technician 1				
Product lot	SC-003				
Sample diluent buffer lot	DIL10-356 (2025-08)				
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012				
Temp. (ºC) / RH (%)	23.5 / 41.1				
Measuring device	EQ-1033				
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	Repeatability (repetitions)				
	1st	2nd	3rd	4th	5th
32.00	+	+	+	+	+
16.00	+	+	+	+	+
8.00	+	+	+	+	+
4.00	+	+	+	+	+
2.00	+	+	+	+	+
1.00	+	+	+	+	+
0.50	±	±	±	-	±
0.25	-	-	-	-	-
0.13	-	-	-	-	-
0.00	-	-	-	-	-

The repeatability found for each of the five replicates of the three analysed lots is better than ± onetwo-fold dilution (± ½).

15.2 Reproducibility Inter-Lot

This measurement has been performed by diluting Coronavirus SARS-CoV-2 antigen in a sample dilution buffer (see the following tables). Employed strip lots were SC-001, SC-002 and SC-003. (Laboratory Technician: Technician 1, date: 03/09/2020)

For the practical realization of the reproducibility inter-lot, each lot of validation three- will be measured 1 time with the same equipment (in this case micropipettes EQ-1849X, 1850X, 1851X) in the same day and performed by the same technician.

Laboratory technician	Technician 1		EQ-1033
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012		Temp. (ºC) / RH (%)
Sample diluent Buffer lot	DIL10-354 (2025-08); 10-355 (2025-08); 10-356 (2025-08)		23.5 / 41.1
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	SC-001	SC-002	SC-003
32.00	+	+	+
16.00	+	+	+
8.00	+	+	+
4.00	+	+	+
2.00	+	+	+
1.00	+	+	+
0.50	+	-	±
0.25	-	-	-
0.13	-	-	-
0.00	-	-	-

The inter-lot reproducibility found for the three analysed lots is better than ± one two-fold dilution (± ½).

15.3 Reproducibility Inter-Operator

Again, this measurement has been performed by diluting Coronavirus SARS-CoV-2 antigen in a sample dilution buffer (see the following tables). The employed strips lots were: SC-001, SC-002 and SC-003. 4 different laboratory technicians.

Coronavirus SARS-CoV-2 antigen (date: 03/09/2020)	
Laboratory Technicians	
T1(*1)	Technician 1 (lots: SC-001, SC-002, SC-003)
T2(*1)	Technician 2 (lots: SC-001, SC-002, SC-003)
T3(*1)	Technician 3 (lots: SC-001, SC-002, SC-003)
T4(*1)	Technician 4 (lots: SC-001, SC-002, SC-003)

(*1) Note: T1: technician 1; T2, Technician 2; T3: Technician 3; T4: Technician 4.

For the practical realization of the reproducibility inter-operator, each lot of validation will be measured 1 time with the same equipment (in this case micropipette EQ-

1849X,1850X,1851X) in the same day and performed by different technician (in this case 4 technicians).

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012				EQ-1033							
Sample diluent Buffer lot	DIL10-354 (2025-08); 10-355 (2025-08); 10-356 (2025-08).				Temp.(ºC)		RH (%)					
Date	03/09/2020				23.5		41.1					
Certest SARS-CoV-2 Test	SC-001				SC-002				SC-003			
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
32.00	+	+	+	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+	+	+	+
0.50	+	+	+	+	-	±	-	-	±	±	±	±
0.25	-	-	-	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-	-	-	-

The reproducibility found by each of the four different operators with the three analyzed lots is better than ± one two-fold dilution (± ½).

15.4 Reproducibility Inter-Day

These measurements have been performed by diluting Coronavirus SARS-CoV-2 antigen in a sample dilution buffer (see the following tables) and employing the strips SC-001, SC-002 and SC-003.

Coronavirus SARS-CoV-2 antigen (technician: Technician 1)	
Days	
D1(*2)	02/09/2020 (lots: SC-001, SC-002, SC-003)
D2(*2)	03/09/2020 (lots: SC-001, SC-002, SC-003)
D3(*2)	04/09/2020 (lots: SC-001, SC-002, SC-003)

(*2) Note: D1: day 1, D2: day 2, D3: day 3.

For the practical realization of the reproducibility inter-day, each lot of validation will be measured 1 time with the same equipment (in this case micropipettes EQ-1849X,1850X,1851X) at three different days and performed by the same laboratory technician.

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012	EQ-1033	D1	D2	D3
Sample diluent Buffer lot	DIL10-354 (2025-08); 10-355 (2025-08); 10-356 (2025-08)	Temp. (ºC)	23.5	23.0	23.5
Laboratory technician	Technician 1	RH (%)	40.0	41.0	40.0

Certest SARS-CoV-2 Test	SC-001			SC-002			SC-003		
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	D1	D2	D3	D1	D2	D3	D1	D2	D3
32.00	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+
0.50	±	+	±	-	-	±	±	±	±
0.25	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-

The reproducibility found during three different running days with the three validated lots is better than ± one two-fold dilution (± ½)

15.5 Limit of Detection

These measurements have been performed by diluting Coronavirus SARS-CoV-2 antigen in the sample dilution buffer (see the following tables) and employing the strip tests SC-001, SC-002 and SC-003.

For the practical realization of the detection limit, three lots will be measured 5 times the with same equipment (in this case micropipettes EQ-1849X,1850X,1851X) at the same day and performed by the same laboratory technician. The detection limit value (target value) will be calculated as the concentration value for which the number of positives will be equal or higher than 12 times positives of the 15 repetitions.

In our case and for our products a probability of 80% is established, since due to the characteristics of the same it is normal that for the same product making several repetitions of sensitivity curves we have variations of the results between them of ± 1/2 of dilution, that is to say, it must be confirmed to what at a given antigen concentration and, at least 12 of the 15 samples

analysed, look positive (80%).

Increasing the percentage is not considered viable since, due to the variation ± 1/2 that there is usually, it may be that of the 15 samples in some cases it could not be fulfi

The detection limit range will be: one dilution or two dilutions before and after of the target value.

When the result is positive (+ or +/-) we indica1; when the result is negative (-), we indicate: 0.

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012	Test Lot	SC-001; SC-002; SC-003	EQ-1033	23.5 ºC	41.1 % RH
Sample diluent buffer lot	DIL10-354 (2025-08); 10-355 (2025-08); 10-356 (2025-08).	Technician	Technician 1	Date		03-09-2020

	1	2	3	4	5	6	7	8	9	B
Concentration (ng/mL)	32.00	16.00	8.00	4.00	2.00	1.00	0.50	0.25	0.13	0.00
R1(*3)	1	1	1	1	1	1	1	0	0	0
R2(*3)	1	1	1	1	1	1	0	0	0	0
R3(*3)	1	1	1	1	1	1	0	0	0	0
R4(*3)	1	1	1	1	1	1	0	0	0	0
R5(*3)	1	1	1	1	1	1	1	0	0	0
R6(*3)	1	1	1	1	1	1	0	0	0	0
R7(*3)	1	1	1	1	1	1	1	0	0	0
R8(*3)	1	1	1	1	1	1	1	0	0	0
R9(*3)	1	1	1	1	1	1	1	0	0	0
R10(*3)	1	1	1	1	1	1	1	0	0	0
R11(*3)	1	1	1	1	1	1	1	0	0	0
R12(*3)	1	1	1	1	1	1	1	0	0	0
R13(*3)	1	1	1	1	1	1	1	0	0	0
R14(*3)	1	1	1	1	1	1	0	0	0	0
R15(*3)	1	1	1	1	1	1	1	0	0	0
Nº negatives	0	0	0	0	0	0	5	15	15	15
Nº positives	15	15	15	15	15	15	10	0	0	0

(*3) Note: R1: repetition 1, 5 U H S H W L W L R Q 5 U H S H W L W L R Q 5 U H S H W L W L R Q « 5 U H

With these results we can determinate the detection limit for Coronavirus SARS-CoV-2 antigen, that is ± one two-fold dilution (± ½).

15.5.1 Limit of Detection calculated with 20 replicates

These measurements have been performed by diluting Coronavirus SARS-CoV-2 antigen in a sample dilution buffer (see the following tables) and employing the strips of SC-001, SC-002 and SC-003.

For the practical realization of the detection limit, three lots will be measured 20 times with the same equipment (in this case micropipettes EQ-1849X,1850X,1851X) and performed by the same laboratory technician. The detection limit value (target value) will be calculated as the concentration value for which the number of positives will be equal or higher than 95% positives of the 20 repetitions. We have repeated this for three lots.

For determine the detection limit of the CerTest SARS-CoV-2 Test have been evaluated three lots (SC-001, SC-002 and SC-003) have been evaluated several antigen concentrations from 32.00 to 0.00 ng/mL of Coronavirus recombinant nucleoprotein (lot C186NP-C012) diluted in the sample diluent (DIL10-354, 355, 356). For each of the three lots have been repeated 20 times these measures.

The detection limit range will be: one dilution or two dilutions before and after of the target value.

Laboratory technician	Technician 1																		
Product lot	SC-001			Sample diluent buffer lot		DIL 10-354 (2025-08)													
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012			Temp. (ºC) / RH (%)		23.5 / 41.1			Measuring device (temperature and humidity)			EQ-1033							
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	repetitions																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
32.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	±	+	+	+
0.50	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Laboratory technician	Technician 1																		
Product lot	SC-002			Sample diluent buffer lot		DIL10-355 (2025-08)													
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012			Temp. (ºC) / RH (%)		23.5/ 41.1				Measuring device (temperature and humidity)		EQ-1033							
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	repetitions																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
32.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.50	-	±	±	±	±	±	±	±	±	-	±	-	-	±	±	±	±	-	±	±
0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Laboratory technician	Technician 1																		
Product lot	SC-003			Sample diluent buffer lot		DIL10-356 (2025-09)													
Coronavirus SARS-CoV-2 antigen lot	C186NP-C012			Temp. (ºC) / RH (%)		23.5/ 41.1				Measuring device (temperature and humidity)		EQ-1033							
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	repetitions																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
32.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.50	±	±	±	-	±	±	±	-	±	±	±	±	±	±	±	±	±	±	±	±

0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

15.5.2 Limit of Detection calculated with culture (TCID50/mL)

Detection limit 1.00 ng/mL of Coronavirus SARS-CoV-2 nucleoprotein antigen, this is the one obtained in the previous LoD asssays. This would be the detection limit calculate with 3 lots and several repetitions (and with 20 replicates procedure repeated for each lot).

These measurements have been performed by dilution Coronavirus SARS-CoV-2 antigen (Zeptometrix culture SARS-CoV-2) in the sample dilution buffer and employing three lots SC-001, SC-002 and SC-003 (see the following table)

The Detection limit in $TCID_{50}$/mL stablished for these three lots is approximately $1 \cdot 10^3 TCID_{50}$/mL, as can be extrapolated from the following table of results:

Laboratory technician	Technician 6	EQ-1033
Coronavirus SARS-CoV-2 lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA-WA1/2020. Lot: 324609	Temp. (ºC) / RH (%)
Sample diluent Buffer lot	DIL10-354 (2025-08); 10-355 (2025-08); 10-356 (2025-08)	23.5 / 41.1

Dilution	$TCID_{50}$/mL	SC-001	SC-002	SC-003
100	15100	+	+	+
200	7550	+	+	+
400	3775	+	+	+
800	1888	+	+	+
1600	944	±	+	+
3200	472	-	-	-
6400	236	-	-	-
0	0	-	-	-

15.5.3 Limit of Detection calculated with culture (TCID50/mL) and 20 replicates

The Detection limit in $TCID_{50}$/mL stablished for these three lots is approximately $1 \cdot 10^3 TCID_{50}$/mL, as can be extrapolated from the following table of results (with 20 replicates):

Laboratory technician	Technician 1				
Product lot	SC-001	Sample diluent buffer lot	DIL 10-354 (2025-08)		
Culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA WA1/2020. 324609	Temp. (ºC) / RH (%)	23.1 / 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1600	944	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	±	+	+	+
3200	472	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Laboratory technician	Technician 1				
Product lot	SC-002	Sample diluent buffer lot	DIL 10-355 (2025-08)		
Culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA WA1/2020. 324609	Temp. (ºC) / RH (%)	23.1 / 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1600	944	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	±	+	+	+
3200	472	-	±	±	±	±	±	±	±	±	-	±	-	-	±	±	±	±	-	±	±
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Laboratory technician	Technician 1	Sample diluent buffer lot	DIL 10-356 (2025-08)		
Product lot	SC-003				
Culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA WA1/2020. 324609	Temp. (ºC) / RH (%)	23.1 / 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1600	944	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	±	+	+	+
3200	472	±	±	±	-	±	±	±	-	±	±	±	±	±	±	±	±	±	±	±	±
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

15.5.4 Limit of Detection calculated with matrix spiked samples and 20 replicates

These measurements have been performed adding Coronavirus SARS-CoV-2 culture to negative nasopharyngeal samples (these negative samples were confirmed as SARS-CoV-2 negative by qPCR technique). The culture was added to obtain the several concentrations evaluated (spiked samples).

Then these samples were processed following the procedure for sample extraction of the IFUS.

For the practical realization of the detection limit, one lot will be measured 20 times with the same equipment (in this case micropipettes EQ-1849X,1850X,1851X) and performed by the same laboratory technician. The detection limit value (target value) will be calculated as the concentration value for which the number of positives will be equal or higher 95% times positives of the 20

repetitions. We have repeated this for one lot.

For determine the detection limit of the CerTest SARS-CoV-2 Test have been evaluated one lot (SC-018) have been evaluated several antigen concentrations (15100 to 0.00 TCID50/mL of Coronavirus culture (Zeptometrix Culture SARS-CoV-2, Isolate: 2019nCoV/USA-WA1/2020. diluted in the sample diluent (DIL10-518).

The detection limit range will be: one dilution or two dilutions before and after of the target value.

Nasopharyngeal swab + sample diluent provided into the kit.

Laboratory technician	Technician 1				
Product lot	SC-018	Sample diluent buffer lot	DIL 10-518 (2025-12)		
Coronavirus SARS-CoV-2 culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA-WA1/2020. 324609	Temp. (ºC) / RH (%)	21.7 / 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1600	944	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3200	472	-	±	±	±	-	-	±	±	±	-	±	-	±	±	±	±	-	±	-	±
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Nasopharyngeal swab preserved in VTM (transport media) + sample diluent provided into the kit. Proportion 1:1

Laboratory technician	Technician 1	VTM (vircell) lot 20TM123 (2022-04)			
Product lot	SC-018	Sample diluent buffer lot	DIL 10-518 (2025-12)		
Coronavirus SARS-CoV-2 culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA-WA1/2020. 324609	Temp. (ºC) / RH (%)	21.7 / 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1600	944	±	±	+	+	+	+	+	+	+	+	±	+	±	+	+	+	+	±	±	±
3200	472	-	±	±	±	-	-	+	±	±	-	-	±	±	±	+	±	-	±	-	±
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Nasopharyngeal swab preserved in UTM (transport media) + sample diluent provided into the kit. Proportion 1:1

Laboratory technician	Technician 1	UTM (Copan) lot 2019867 (2022)			
Product lot	SC-018	Sample diluent buffer lot	DIL 10-518 (2025-12)		
Coronavirus SARS-CoV-2 culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA-WA1/2020. 324609	Temp. (ºC) / RH (%)	21.7 / 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1600	944	±	+	+	+	+	±	+	+	+	±	±	±	+	+	+	+	+	±	+	+
3200	472	-	+	±	±	-	-	+	±	±	-	-	-	±	±	+	±	-	±	-	±
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Nasopharyngeal swab preserved in Saline Buffer (transport media) + sample diluent provided into the kit. Proportion 1:1

Laboratory technician	Technician 1	Saline Buffer (BS), lot ET82-171 (2021-11)			
Product lot	SC-018	Sample diluent buffer lot	DIL10-518 (2025-12)		
Coronavirus SARS-CoV-2 culture lot	Zeptometrix Culture SARS-CoV-2 Isolate: 2019nCoV/USA-WA1/2020. 324609	Temp. (ºC)/ RH (%)	21.7/ 43.2	Measuring device (temperature and humidity)	EQ-1033

Dilution	Coronavirus SARS-CoV-2 Culture (TCID50/mL)	Repetitions																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
100	15100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	7550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	3775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	1888	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1600	944	±	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	±	+	+
3200	472	-	±	±	±	-	±	+	±	±	-	-	±	±	±	+	±	-	±	-	±
6400	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The LoD for the evaluated lot and conditions (direct sample from nasopharyngeal swab (only extracted with sample diluent provided into the kit, sample extracted in UTM and sample diluent (1:1) sample extracted in VTM and sample diluent (1:1) and sample extracted in saline buffer and sample diluent (1:1) is $1 \cdot 10^3$ $TCID_{50}$/mL.

15.6 Analytical Specificity

Certest SARS-CoV-2 Test recognizes/detects the following antigens / species:

- Coronavirus SARS-CoV-2 antigen.

SARS-CoV-2 Test has some cross reaction with SARS (Severe Acute Respiratory Syndrome) and very low with MERS (Middle East Respiratory Syndrome):

- SARS: ± 1 µg/mL of SARS-1 recombinant nucleoprotein, lot: SARS-004 for product lots (SC-001, SC-002 and SC-003).
- MERS: ± 5 µg/mL MERS-CoV recombinant nucleoprotein, lot: MERS-003 for product lots (SC-001, SC-002 and SC-003).
  MERS: - (negative) $8.50 \cdot 10^{4}$ $TCID_{50\%}$ of Zeptometrix MERS-CoV, Strain Florida/USA-2-Saudi Arabia.2014 (1/2 Dil.) lot: 324609 for product lots (SC-001, SC-002 and SC-003).

Human coronavirus HKU1 has not been tested from culture (*). For this reason, we cannot assure that cross-reaction with Human coronavirus HKU1 could not exit, even though the percentage identity of the nucleocapsid protein sequence of HKU1 with the nucleocapsid protein sequence of SARS-CoV-2 is about 35% that is considered as very low homology.

(*) Five positive samples on HKU1 were tested in an external evaluation performed to the CerTest SARS-CoV-2 in Amiens (France), October 2020 and the result obtained was negative. So this could means that there is no cross reactivity with HKU1.

Three positive samples for Rhinovirus were tested in an external evaluation performed to the CerTest SARS-CoV-2 in Amiens (France), October 2020 and the result obtained was negative. So this could means that there is no cross reactivity with Rhinovirus.

Certest SARS-CoV-2 Test does not recognizes/detects the following antigens / species:

- Other Coronavirus strains such as 229E, NL63 and OC43.

See point 15.9 (Cross Reactivity Studies) to know more about the specimens that do not react with this strip.

15.7 Stability Assay

The aim of this stability assay is to demonstrate that the characteristics and performance of the Certest SARS-CoV-2 Test is not altered with the time under normal use due to the influence of different environmental factors such as: temperature, humidity, transport conditions, sunlight or others that could modify the proper performance of the test.

To cover these requirements and could set the stability and expiry date of test a global evaluation has been done taking into account extreme conditions (accelerate ageing) and real-time ageing after expiry date.

Other conditions that could affect to the product have been evaluated as shipping conditions and the stability in use.
This evaluation has been performed by CerTest BioTec (Zaragoza, SPAIN) following the internal protocol.

The materials that compose our products (lateral flow test) could suffer alterations with time, depending on transport and storage conditions.

The aim of the experiment is to establish the limit conditions of temperature and expiration date in order to detect how extreme conditions affect the test.

15.7.1 Accelerated Ageing Study

CertestSARS-CoV-2 Test has been designed to be stored at room temperature during the whole validity period. The recommended store temperature is between 2 and 30°C.

To check the tests performance after certain period under extreme temperatures, some few reaction strips from three different lots, and the sample dilution buffer, were exposed to a temperature of 45ºC for 5 days. After that, the devices are used to determine the influence of ageing on the sensibility curve against an antigen reference standard preparation and negative samples will be evaluated with these test strips.

From data gathered developing other immunochromatographic test we have established some correlation between this 45ºC stress and longevity of the device. These conditions are equivalent to 24 months at RT (2-30ºC) conditions. (See equivalence on the report: RT-Accelerated ageing EN).

As most of materials and reagents are the same or very similar, we can conclude that these devices will be stable at least 24 months.

Accelerate conditions	After 5 days at 45ºC	After 5 days at 45ºC	After 5 days at 45ºC
Accelerate conditions 45ºC (±1ºC), Oven EQ-391	Test lot: SC-001 Buffer lot: DIL10-357 (2025-08) Coronavirus SARS-CoV-2 lot: C186NP-C012	Test lot: SC-002 Buffer lot: DIL10-357 (2025-08) Coronavirus SARS-CoV-2 lot: C186NP-C012	Test lot: SC-003 Buffer lot: DIL10-357 (2025-08) Coronavirus SARS-CoV-2 lot: C186NP-C012
Laboratory technician	Technician 7 (EQ-1307, EQ-1308, EQ-1309)	Technician 7 (EQ-1307, EQ-1308, EQ-1309)	Technician 7 (EQ-1307, EQ-1308, EQ-1309)
Temperature (ºC) (EQ-1033)	21.8	21.8	21.8
Humidity (%) (EQ-1033)	76.5	76.5	76.5

Samples prepared by direct dilution of Coronavirus SARS-CoV-2-antigen in the buffer were tested at day 0 (room temperature) and after stress (accelerate conditions: 5 days at 45ºC).

Coronavirus SARS-CoV-2 antigen	Accelerated Ageing					
	SC-001 (DIL 10-357)		SC-002 (DIL 10-357)		SC-003 (DIL 10-357)	
ng/mL	Day 0 18.09.20	Stress conditions	Day 0 18.09.20	Stress conditions	Day 0 18.09.20	Stress conditions
32.00	+	+	+	+	+	+
16.00	+	+	+	+	+	+
8.00	+	+	+	+	+	+
4.00	+	+	+	+	+	+
2.00	+	+	+	+	+	+
1.00	±	+	±	±	±	±
0.50	-	-	-	-	-	-
0.25	-	-	-	-	-	-
0.13	-	-	-	-	-	-
0.00	-	-	-	-	-	-

There are no significant differences between the three lots tested. All the results fulfil the expected values: 0.50 to 4.00 ng/mL of Coronavirus SARS-CoV-2 antigen.

At the same time, a study of functionality is established after the evaluation of each batch: several representative negative samples to see if false positive appear. (The samples have been previously confirmed as negative for Coronavirus SARS-CoV-2 antigen). This study is carried out at day 0 and after the accelerated study.

Nº Sample	Evaluation of negatives samples at day 0 (RT)			Nº Sample	Evaluation of negatives samples after stress conditions		
	SC-001	SC-002	SC-003		SC-001	SC-002	SC-003
1	-	-	-	1	-	-	-
2	-	-	-	2	-	-	-
3	-	-	-	3	-	-	-

There are not any false positive results.

As data show, Certest SARS-CoV-2 Test maintain its properties even to stand extreme temperature conditions.

It is concluded that the result for the Accelerated Ageing assay, has been better than a ± one-fold dilution ½ with respect to its evaluation at day 0. The Certest SARS-CoV-2 Test resists stress conditions (5 days by at 45ºC) without a significant modification of its performance.

15.7.2 Real Time Stability Data (RTSD)

These measurements are performed by diluting the Coronavirus SARS-CoV-2 antigen in a sample dilution buffer. This series will be repeated for three lots. The strips and the sample dilution buffer will be previously exposed to various conditions: room temperature (15-25ºC), fridge (4±3ºC) and oven (30±1ºC), in order to cover the entire temperature range at which the IC-Test product could be preserved as indicated in its labels.

Several measures of these three preservation conditions will be taken at different time intervals: 0 months (day 0), 3 months, 6 months, 12 months, 18 months and 25 months, in order to cover the entire expiration date of the IC-Test, which is 24 months from the date of manufacture.

In order to perform the assay to set the lifetime of the product, the products assayed were conserved in the primary packaging (thermosealed aluminium bag, pouch)[*4] with silica gel as desiccant that preserved them form the humidity.

At the same time that the different measures of the diluted antigens in the sample buffer are carried out, at the different conditions, several negatives samples will be evaluated in order to determine if exist the possibility to obtain false positive results after exposing the test to the different conditions evaluated. (The samples have been previously confirmed as negative for Coronavirus SARS-CoV-2 antigen). This study will be carried out on day 0 and after each of the time and temperature conditions studied.

The assay of Certest SARS-CoV-2 Test will be performed in the following months and the results will be exposed at this report. This is a new launch and the Stability Assay Real Ageing will be completed until 25 months. At this moment, we have the stability data for day 0 and 3 months. The real stability will be done when these three lots reach the 6, 12, 18 and 25 months since manufacture.

[*4] Note: there are no significant variations between different primary containers (pouches, blister, tube).

Real ageing	Day 0	Day 0	Day 0
Room Temperature	Test lot: SC-001 Buffer lot: DIL10-357 (2025-08) Coronavirus SARS-CoV-2 lot: C186NP-C012	Test lot: SC-002 Buffer lot: DIL10-357 (2025-08) Coronavirus SARS-CoV-2 lot: C186NP-C012	Test lot: SC-003 Buffer lot: DIL10-357 (2025-08) Coronavirus SARS-CoV-2 lot: C186NP-C012
Laboratory technician	Technician 7 (EQ-1307, EQ-1308, EQ-1309)	Technician 7 (EQ-1307, EQ-1308, EQ-1309)	Technician 7 (EQ-1307, EQ-1308, EQ-1309)
Temperature (ºC) (EQ-1033)	21.6	21.6	21.6
Humidity (%) (EQ-1033)	76.3	76.3	76.3

Real ageing	3 Months	3 Months	3 Months
Room Temperature	Test lot: SC-001 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)	Test lot: SC-002 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)	Test lot: SC-003 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)
Laboratory technician	Technician 4 (EQ-785, EQ-783, EQ-784)	Technician 4 (EQ-785, EQ-783, EQ-784)	Technician 4 (EQ-785, EQ-783, EQ-784)
Temperature (ºC) (EQ-1033)	22.7	22.7	22.7
Humidity (%) (EQ-1033)	47.2	47.2	47.2
Fridge (4ºC +/-3)	Test lot: SC-001 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)	Test lot: SC-002 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)	Test lot: SC-003 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)
Laboratory technician	Technician 4 (EQ-785, EQ-783, EQ-784)	Technician 4 (EQ-785, EQ-783, EQ-784)	Technician 4 (EQ-785, EQ-783, EQ-784)
Temperature (ºC) (EQ-1033)	22.7	22.7	22.7
Humidity (%) (EQ-1033)	47.2	47.2	47.2
Owen (30ºC +/-1)	Test bt: SC-001 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)	Test lot: SC-002 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)	Test lot: SC-003 Buffer lot: DIL10-356 (2025-08) Coronavirus SARS-CoV-2 lot: AgCV-00002 (C186NP- C002)
Laboratory technician	Technician 4 (EQ-785, EQ-783, EQ-784)	Technician 4 (EQ-785, EQ-783, EQ-784)	Technician 4 (EQ-785, EQ-783, EQ-784)
Temperature (ºC) (EQ-1033)	22.7	22.7	22.7
Humidity (%) (EQ-1033)	47.2	47.2	47.2

0 MONTHS at room temperature (RT)

Coronavirus SARS-CoV2 antigen	Real ageing		
	SC-001	SC-002	SC-003
ng/mL	Day 0 18.09.20	Day 0 18.09.20	Day 0 18.09.20
32.00	+	+	+
16.00	+	+	+
8.00	+	+	+
4.00	+	+	+
2.00	+	+	+
1.00	±	±	±
0.50	-	-	-
0.25	-	-	-
0.13	-	-	-
0.00	-	-	-

3 negative samples for SARS-CoV-2 were tested at time 0 and room temperature. The samples have been previously confirmed as negative for SARS-CoV-2.

There are not any false positive results.

Nº Sample	Evaluation of negatives samples at day 0 (RT)		
	SC-001	SC-002	SC-003
1	-	-	-
2	-	-	-
3	-	-	-

3 MONTHS at room temperature (RT), Fridge (4ºC+/-3) and Owen (30ºC+/-1)

Coronavirus SARS-CoV-2 antigen ng/mL	Real Time Stability								
	SC-001	(DIL 10-356)		SC-002	(DIL 10-356)		SC-003	(DIL 10-356)	
	Day 0 18.09.20 (RT)	Day 0 18.09.20 (fridge)	Day 0 18.09.20 (oven)	Day 0 18.09.20 (RT)	Day 0 18.09.20 (fridge)	Day 0 18.09.20 (oven)	Day 0 18.09.20 (RT)	Day 0 18.09.20 (fridge)	Day 0 18.0920 (oven)
32.00	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+
2.00	±	±	±	±	±	±	±	±	±
1.00	-	-	-	-	-	-	-	-	-
0.50	-	-	-	-	-	-	-	-	-
0.25	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-

4 negative samples for SARS-CoV-2 were tested at time 3 months (at room temperature, Fridge (4ºC+/-3) and Owen (30ºC +/-1). The samples have been previously confirmed as negative for SARS-CoV-2.

There are not any false positive results

Nº Sample	Evaluation of negatives samples at 3 months (RT)			Evaluation of negatives samples at 3 months (Fridge)			Evaluation of negatives samples at 3 months (Owen)		
	SC-001	SC-002	SC-003	SC-001	SC-002	SC-003	SC-001	SC-002	SC-003
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-

15.7.3 Stability in transport

In order to perform a correct Transport Stability Assay, the temperature and humidity conditions that the product could experience during a standard shipment must be established. For this reason, several data loggers were sent along with the final product, and various types of shipping were studied (the main shipping conditions that are carried out in CerTest). The place chosen were 4: South Africa (plane), Ecuador (plane), Uruguay (ship) and Saudi Arabia (plane). The delivery time of these shipments was determined as an average of 8 days: a total duration of 8 hour supporting temperatures between -5ºC until 45ºC was the determined as temperatures cycles that our products are supporting during a standard trip.

After determining the conditions of the simulated transport, several lots for IC-Test were committed to this cycle in a climatic chamber, Dycometal (code 2PG50052-136) (ITA)

After these cycles of temperature, the product (one lot) will be evaluated (sensitivity) in or

to see if his performance or results could be affected for this.

The lots were tested in parallel. One kit was preserved at Certest facilities at room temperature and the same kit (same lot and catalogue reference) was send to the tandem ITA submitted to this cycle of temperatures.

In this case, the measurements have been repeated for the 3 validation lots prepared: SC-001, SC-002 and SC-003.

(*5) Note: For more information about this assay, see document, Instituto Tecnológico de Aragón, Assay report C201999I1, September 2020.

Coronavirus SARS-CoV-2	t (0): C186NP-C012 After shipping test: AgCV-00002 (C186NP- C002)	Test Lot	SC-001; SC-002; SC-003	EQ-1033	After shipping test: Tª (ºC): 21.2 RH (%): 52.3
Sample diluent buffer lot	t (0): DIL10-351 (2025-08) After shipping test: DIL10-376 (2025-09)	Technician	t (0): Technician 4 (EQ-1304, EQ-1305, EQ-1306) After shipping: Technician 4 (EQ-1304, EQ-1305, EQ-1306)	Date	t (0): 02-09-2020 After shipping test: 08-10-2020

Certest SARS-CoV-2 Test		1	2	3	4	5	6	7	8	9	B
Concentration (ng/mL)		32.00	16.00	8.00	4.00	2.00	1.00	0.50	0.25	0.13	0.00
SC-001	Sensitivity assessment t0	+	+	+	+	+	±	-	-	-	-
	Sensitivity assessment Af. Ship. test	+	+	+	+	+	-	-	-	-	-
SC-002	Sensitivity assessment, t0	+	+	+	+	+	±	-	-	-	-
	Sensitivity assessment, Af. Ship. test	+	+	+	+	+	-	-	-	-	-
SC-003	Sensitivity assessment t0	+	+	+	+	+	±	-	-	-	-
	Sensitivity assessment Af. Ship. test	+	+	+	+	+	±	-	-	-	-

There are no significant differences between the three lots tested. All the results fulfil the expected values: 0.50 to 4.00 ng/mL of Coronavirus SARS-CoV-2 antigen.

At the same time, a study of functionality is established after the evaluation of the batch.

Several representative negative samples are tested to see if false positive appear. (The samples have been previously confirmed as negative for Coronavirus SARS-CoV-2 antigen). This study is carried out at day 0 and after the stability in transport study.

Evaluation of negatives samples at day 0 (RT)		Evaluation of negatives samples after Stability in Transport Assay	
Nº sample	Evaluation	Nº sample	Evaluation
1	-	1	-
2	-	2	-
3	-	3	-
4	-	4	-
5	-	5	-
6	-	6	-
7	-	7	-
8	-	8	-
9	-	9	-
10	-	10	-

There are not any false positive results.

As data show, Certest SARS-CoV-2 Test maintain its properties within shipment condition.

It is concluded that the result for the stability in transport evaluation of Certest SARS-CoV-2 Test has been equal or better than a ± one-fold dilution with respect to its evaluation at day 0. The Certest SARS-CoV-2 Test resists these conditions without a significant modification of its performance.

15.7.4 Stability in use

To determine the stability in use of the Certest SARS-CoV-2 Test, a few reaction strips from one lot (SC-001) were exposed at room temperature and evaluated at time 0 (0 months). The pouches were opened and evaluated (sensitivity curve) at different interval of times: 0, 5, 10, 15, 30 and 120 minutes.

The assay consists of preparing several dilutions of Coronavirus SARS-CoV-2 antigen in the sample dilution buffer and these dilutions are tested after opening the primary container at the different time intervals. With this assay we can evaluate how it could affect humidity to test, once its primary container is open.

The components of the kit, test strips, are for only one use so no additional assays for the stability in use are required.

Date	18/09/2020		Test lot		SC-001	
Laboratory Technician	Technician 7 (EQ-1307, EQ-1308, EQ-1309)		Sample buffer lot		DIL10-357 (2025-08)	
Measuring device code	LAB-3-66		Coronavirus SARS-CoV-2 antigen lot		C186NP-C012	
Time (min) since opening the primary container	0	5	15	30	60	120
Temperature (ºC)	21.6	22.1	22.4	22.4	22.4	21.8
Humidity (%)	76.3	77.8	79.2	78.7	76.1	76.5

Stability in use, Room Temperature						
Certest SARS-CoV-2 Test	SC-001					
Coronavirus SARS-CoV-2 concentration (ng/mL)	0 min	5 min	15 min	30 min	60 min	120 min
64.00	+	+	+	+	+	+
32.00	+	+	+	+	+	+
16.00	+	+	+	+	+	+
8.00	+	+	+	+	+	+
4.00	+	+	+	+	+	+
2.00	+	+	+	+	+	+
1.00	±	±	±	±	±	±
0.50	-	-	-	-	-	-
0.25	-	-	-	-	-	-
0.13	-	-	-	-	-	-
0.00	-	-	-	-	-	-

There are no significant differences between the different time intervals tested. All the results fulfil the expected values: 0.50 to 4.00 ng/mL of Coronavirus SARS-CoV-2 antigen.

As data show, Certest SARS-CoV-2 Test maintain its properties within after opening the primary container for, at least, 120 min later.

It is concluded that the result for the stability in use evaluation of the Certest SARS-CoV-2 Test has been equal or better than a ± one-fold dilution ½ with respect to its evaluation at day 0. The Certest SARS-CoV-2 Test resists these conditions without significant modification of its performance.

The stability in use for the sample diluent has been tested for opening the sample diluent bottle for at least 22 times and there is not any change on it. This seem not affect to the stability in use Respiratory sample diluent.

15.8 Prozone / Hook Effect

When performing immunoassays, the concentration of the antigen and the titter of the antibody must be considered. An antigen concentration in a sample below the sensitivity of detection of the assay will yield a negative result. A negative result also may be obtained when too high a concentration of antigens is assayed. The latter situation is known as high dose hook effect. This effect occurs in several immunoassays with antigen-antibody reaction.

In these systems, antigen in excess will overwhelm the mobile monoclonal antibodies and unbound antigens will migrate towards the reaction zone, where the immobilized monoclonal antibodies reside. Free unbound antigen binds to the immobilized antibodies and prevents these antibodies from reacting with the antigen-mobile antibody complexes. The result will be a false negative.

The purpose of this section is to determine how high dose hook effect affects to Certest SARS-CoV-2 Test.

The materials and conditions under which this test was carried out, on 2nd September 2020, are summarized in the following lines:

- Environmental Conditions: 23.5 ºC and 41.0 % RH.
- IC Reader: Cube reader EQ-842.
- Laboratory Technician: Technician 1 (EQ-1849X,1850X,1851X)

Components	Lot	Exp. Date
Certest SARS-CoV-2 Test	SC-002	2022-08
Diluent	DIL 10-354	2025-08
Antigen Preparation	C186NP-C012	NA

For determining the high dose hook effect, we prepared and analyse a wide range of antigen concentrations diluted in the sample diluent provided in the kit. The obtained results were:

Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	Certest SARS-CoV-2 Test (SC-002)	LF Reader peak height
202500.00	+	105.23
101250.00	+	286.25
50625.00	+	345.32
25312.50	+	359.86
12656.25	+	737.29
6328.13	+	978.14

3164.06	+	1010.75
1582.03	+	816.93
791.02	+	1086.17
395.51	+	1159.33
197.75	+	1141.74
98.88	+	1065.83
49.44	+	847.38
24.72	+	622.64
12.36	+	277.94
6.18	+	133.47
3.09	+	14.75
1.54	±	15.56
0.77	-	0.00
0.39	-	0.00
0.00	-	0.00

Very high concentrations of Coronavirus SARS-CoV-2 antigen were tested without observing any decrease in the intensity of the positive signals (visual interpretation). The higher concentration values (higher than the maximum values that can be found among the population) was 202500.00 ng/mL for Coronavirus SARS-CoV-2 antigen, about 100000-fold its detection limit (or 100-fold its LoD, expressed as 2019 n-CoV/USA-WA1/2020).

The test for SARS-CoV-2 do not show full inhibitory prozone / Hook effect up to nucleoprotein antigen concentrations as high as 0.2 mg/mL. This concentration is much higher than

the maximum antigen concentration expected in physiological samples.

15.9 Flex and robustness studies

15.9.1 Sample diluent volume

Sample diluent assay to determine the best volume for sample extraction.

The recommended volume of sample diluent to get extracted the sample from the sterile swab is validated and indicate in the protocol of the Instructions for use of the product as (500µL card) and 670µL (for the quad format).

We have evaluated an interval of sample diluent volume and these different volumes (400, 500, 600, 700 and 800µL) have been tested by triplicate with lot number SC-011 (2022-10) and with sample diluent lot number: DIL10-406 (2025-10).

The following table show the results:

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012	EQ-1033	400	500	600	700	800
Sample diluent Buffer lot	DIL10-406 (2025-10)	Temp. (ºC)	21.0	21.0	21.0	21.0	21.0
Laboratory technician	Technician 1	RH (%)	24.0	24.0	24.0	24.0	24.0

Certest SARS-CoV-2 Test	SC-011														
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)*	Volume Sample diluent														
	400µL	400µL	400µL	500µL	500µL	500µL	600µL	600µL	600µL	700µL	700µL	700µL	800µL	800µL	800µL
64.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.50	+	+	+	±	±	±	±	±	±	-	-	-	-	-	-
0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note* concentration when diluting the sample with 500 µL.

Procedure, prepare the sample extraction adding 400, 500, 600, 700 and 800µL of sample diluent, then the antigen is added to obtain the antigen concentrations of the table, then approx. 100µL of the sample are taken and added to the sample window, results read at 10 minutes.

After the assays we can conclude that there are no important differences between adding 400 to 800µL. Our recommendation is to add approx. 500µL of diluent sample into the tube for sample extraction. This should be approx. 15 drops from the sample reagent bottle (500µL). This is for the simple format. When the product is a quad product (CerTest SARS-CoV-2+Flu A +Flu B +RSV) it is necessary to prepare the sample using more volume, the recommended volume then is 670µL (approx. 700 µL). Enough volume to extract the sample properly and to add the recommended volume in each sample window.

15.9.2 Sample volume

Sample volume assay to determine the best volume for adding into sample window.

The recommended volume of sample to add to the window sample has been validated and indicate in the protocol of the Instructions for use of the product as 3 drops.

We have evaluated an interval of sample volume and these different volumes (70, 100 and 150µL) have been tested by triplicate with lot number SC-019 (2022-11) and with sample diluent lot number: DIL10-406 (2025-10).

The following table show the results:

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012			EQ-1033			70	100	150
Sample diluent Buffer lot	DIL10-406 (2025-10)			Temp. (ºC)			23.0	23.0	23.0
Laboratory technician	Technician 1			RH (%)			43.0	43.0	43.0

Certest SARS-CoV2 Test	SC-019			SC-019			SC-019		
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	70µL	70µL	70µL	100µL	100µL	100µL	150µL	150µL	150µL
64.00	+	+	+	+	+	+	+	+	+
32.00	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+
0.50	±	±	±	±	±	±	±	±	±
0.25	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-

Procedure, different SARS-CoV-2 antigen concentrations (showed in the table) were prepared using the sample diluent. Then 70, 100 and 150µL of each of these preparations (different concentrations) are taken and added to the sample window by triplicate, results will be read at 10 minutes.

After the assays we can conclude that there are no important differences between adding 70, 100 or 150µL to the sample window. The volume to be added should be approx. 100µL of sample into the window sample of the cassette. This should be 3 drops from the pipette (100-120µL).

15.9.3 Reading time

Reading time assay to determine the best time for reading the results from the results window

The recommended reading time has been validated and indicate in the protocol of the Instructions for use of the product as 10 minutes since the sample is disposed into sample window.

We have evaluated an interval of reading times and these different times (8, 10 and 12 minutes) have been tested by triplicate with lot number SC-019 (2022-11) and with sample diluent lot number: DIL10-406 (2025-10).

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012			EQ-1033			8	10	12
Sample diluent Buffer lot	DIL10-406 (2025-10)			Temp. (ºC)			22.0	22.0	22.0
Laboratory technician	Technician 1			RH (%)			28.0	28.0	28.0
Certest SARS-CoV-2 Test	SC-019			SC-019			SC-019		
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	8min	8min	8min	10min	10min	10min	12min	12min	12min
64.00	+	+	+	+	+	+	+	+	+
32.00	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+
0.50	±	±	±	±	±	±	±	±	±
0.25	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-

Procedure, different SARS-CoV-2 antigen concentrations (showed in the table) were prepared using the sample diluent. Then 100µL of each of these preparations (different concentrations) are taken and added to the sample window by triplicate, results will be read at 8, 10 and 12 minutes.

After the assays we can conclude that there are no important differences between reading the results at 8, 10 or 12 minutes after adding the sample into the window sample. The results should be read at 10 minutes after dispensing the sample into the sample window.

15.9.4 Temperature to carry out the assay

Temperature when carry out the assay to determine the best temperature (range) for carry out the test.

The recommended temperature for carry out the assay has been validated and indicated in the protocol of the Instructions for use of the product as room temperature (15-30ºC).

We have evaluated an interval of temperatures tested at different times (15, 20 and 30 ºC) have been tested by triplicate with lot number SC-019 (2022-11) and with sample diluent lot number: DIL10-406 (2025-10).

Coronavirus SARS-CoV-2 antigen lot	C186NP-C012	EQ-1033	30	20	15
Sample diluent Buffer lot	DIL10-406 (2025-10)	Temp. (ºC)	30.0	20.0	15.0
Laboratory technician	Technician 1	RH (%)	30.0	28.0	62.5

Certest SARS-CoV-2 Test	SC-019			SC-019			SC-019		
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	30ºC	30ºC	30ºC	20ºC	20ºC	20ºC	15ºC	15ºC	15ºC
64.00	+	+	+	+	+	+	+	+	+
32.00	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+
0.50	±	±	±	±	±	±	±	±	±
0.25	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-

Procedure, different SARS-CoV-2 antigen concentrations (showed in the table) were prepared using the sample diluent. Then 100µL of each of these preparations (different concentrations) are taken and added to the sample window by triplicate, results will be read at 10 minutes.

The temperature conditions of these assays were: 15ºC, 20ºC and 30ºC.

After the assays we can conclude that there are no important differences between carry out the assay at 30, 20 or 15ºC. The test should be carried out at room temperature (and let all the components reach this temperature (15-30ºC). There are no significant differences at this

temperature range.

15.9.5 Humidity (% HR) and light.

Humidity and light when carry out the assay to determine the best humidity (range) and carry out the test and the light for reading and interpretation of the results.

The product is protected from the humidity in the primary container, properly sealed (aluminium pouch or blister) and additionally inside there is silica gel material to preserve the product from the humidity.

Once the primary container is opened the test should be carried out until 2 hours.

We are going to test several humidity ranges (%HR) and we read the results using different types of light in order to determine it these two factors could affect to the product performance or reading results.

We have evaluated variations in humidity and these different humidity levels (20.5%HR (22.9ºC); 45%HR (19ºC) and 75.5%HR (25ºC)) have been tested by triplicate with lot number SC-018 (2022-11) and with sample diluent lot number: DIL10-518 (2025-12).

For each of these levels of humidity the results were reading at different types (levels) of light: natural light (1), fluorescent light (2) and light bulb (3).

Coronavirus SARS-CoV-2 antigen lot	C186NP-C009	EQ-1033	20.5	45	75.5
Sample diluent Buffer lot	DIL10-518 (2025-12)	Temp. (ºC)	22.9	19.0	25.0
Laboratory technician	Technician 1	RH (%)	20.5	45.0	75.5

Certest SARS-CoV-2 Test	SC-018			SC-018			SC-018		
Coronavirus SARS-CoV-2 antigen concentration (ng/mL)	20.5% HR. Light (1)	20.5% HR. Light (2)	20.5% HR. Light (3)	45.0% HR. Light (1)	45.0% HR. Light (2)	45.0% HR. Light (3)	75.5% HR. Light (1)	75.5% HR. Light (2)	75.5%HR. Light (3)
64.00	+	+	+	+	+	+	+	+	+
32.00	+	+	+	+	+	+	+	+	+
16.00	+	+	+	+	+	+	+	+	+
8.00	+	+	+	+	+	+	+	+	+
4.00	+	+	+	+	+	+	+	+	+
2.00	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+
0.50	+	+	+	+	+	+	+	+	+

0.25	-	-	-	-	-	-	-	-	-
0.13	-	-	-	-	-	-	-	-	-
0.00	-	-	-	-	-	-	-	-	-

Procedure, different SARS-CoV-2 antigen concentrations (showed in the table) were prepared using the sample diluent. Then 100µL of each of these preparations (different concentrations) are taken and added to the sample window by triplicate, results were read at 10 minutes.

The humidity conditions of these assays were: 20.5%HR, 45.0%HR and 75.5%HR.

The results were read after 10 minutes under different types of light.

After the assays we can conclude that there no important differences between carry out the assay at different humidity (studied range: 20 to 75%HR) and the different types of light does not imply any important change, it is important a proper light but between the studied there are no differences. There are no significant differences at this humidity range and using several types of light.

15.10 Cross Reactivity Studies

A study of Certest SARS-CoV-2 Test was performed to determine the cross reactivity with common respiratory pathogens, other organisms and substances occasionally present in nasopharyngeal samples.

This evaluation was made in our facilities (CerTest Biotec S.L.) by a CerTest Laboratory Technician, technician 1, on 31st August 2020.

We have prepared different dilutions from the original source of each of the organism (culture or recombinant protein tested) using the sample diluent. In general, elected concentrations (indicated in the following table) were high enough for excluding a significant cross reaction and at least 100 times higher than the normal cut-off of the current lateral flow CerTest tests.

The measurements have been repeated for the 3 validation lots prepared: SC-001 to SC-003.

	Component	Lot	Expiry date
Test	Certest SARS-CoV-2 Test	SC-001, SC-002, SC-003	2022-08
Buffer	DIL10	DIL10-354, DIL10-355, DIL10-356	2025-08
Antigens	Culture/recombinant proteins	See information on table 2	NA

Table 1. Certest SARS-CoV-2 Test lot and sample diluent lot used in this study.

Results of Certest SARS-CoV-2 Test cross reactivity are summarized in the following Table (Table 2).

Interpretation: Positive: Cross Reactivity; Negative: Non cross reactivity.

Microorganism	Strain/Type	Lot number	Concentration	Result
Adenovirus	Type 5 Hexon protein	AgA-00003	$1.17 \cdot 10^{3}$ ng/mL	Negative
Astrovirus	Capsid recombinant protein	AgAT-00004	$3.12 \cdot 10^{3}$ ng/mL	Negative
Calprotectin	HCT	AgHCT-00004	$5.00 \cdot 10^{3}$ ng/mL	Negative
Campylobacter	Jejuni	AgCA-010	$6.28 \cdot 10^{8}$ CFU/mL	Negative
Clostridium difficile Ag	GDH	AgGD-00004	$4.00 \cdot 10^{2}$ ng/mL	Negative
Clostridium difficile Tox	Purified Toxin A	TXAE-C0008	$2.00 \cdot 10^{3}$ ng/mL	Negative
Clostridium difficile Tox.	Purified Toxin B	AgTB-00004	$7.80 \cdot 10^{3}$ ng/mL	Negative
Coronavirus	229E recombinant nucleoprotein	229E-C004	$1.00 \cdot 10^{5}$ ng/mL	Negative
Coronavirus	Coronavirus (Strain: NL63) Culture	323997	$5.85 \cdot 10^{4}$ TCID/mL	Negative
Coronavirus	Betacoronavirus 1; Strain: OC43	ATCC-VR-1558 (70034234)	$4.45 \cdot 10^{5}$ TCID/mL	Negative
Cryptosporidium	Parvum	AgK-012	Dil. 1/200 mL	Negative
Entamoeba	Recombinant protein EH29 histolytica	AgEH-00005	$1.56 \cdot 10^{3}$ ng/mL	Negative
Escherichia coli	O:157	AgE-00002	$1.87 \cdot 10^{8}$ CFU/mL	Negative
Giardia lamblia	Alfa 1 giardin	AgA1G-00006	$6.05 \cdot 10^{2}$ ng/mL	Negative
Giardia lamblia	CPW1	AgGP-00001	$7.80 \cdot 10^{2}$ ng/mL	Negative
Haemoglobin	Human	AgF-00001	$5.00 \cdot 10^{3}$ ng/mL	Negative
Haemoglobin	Pig	090K7609	$5.00 \cdot 10^{4}$ ng/mL	Negative
Haemoglobin	Bovine	10K7618	$5.00 \cdot 10^{4}$ ng/mL	Negative
Helicobacter pylori	Culture	AgP-018	$3.13 \cdot 10^{6}$ CFU/mL	Negative
Influenza A	Recombinant nucleoprotein	AgY-00007	$6.25 \cdot 10^{3}$ ng/mL	Negative

Microorganism	Strain/Type	Lot number	Concentration	Result
Influenza B	Recombinant nucleoprotein	AgYB-00005	$2.50 \cdot 10^{3}$ ng/mL	Negative
Lactoferrin	From human milk	AgLF-00002	$1.00 \cdot 10^{4}$ ng/mL	Negative
Legionella	Pneumophila (Ag)	A106FGF	$6.23 \cdot 10^{3}$ ng/mL	Negative
Listeria	Monocytogenes	AgLM-00006-03	$3.90 \cdot 10^{5}$ CFU/mL	Negative
Norovirus GI	GI.1	14012301	$4.45 \cdot 10^{3}$ ng/mL	Negative
Norovirus GII	GII.4	AgNP-00003	$5.20 \cdot 10^{1}$ ng/mL	Negative
Rotavirus	VP6	AgRP-00003	$1.56 \cdot 10^{3}$ ng/mL	Negative
Respiratory Sincitial Virus	RSV3MHis	AgRVP-00006	$1.00 \cdot 10^{4}$ ng/mL	Negative
Salmonella	Enteritidis	AgSE-00007	$6.25 \cdot 10^{9}$ CFU/mL	Negative
Salmonella	Paratyphi	AgSPA-002	$2.00 \cdot 10^{8}$ CFU/mL	Negative
Salmonella	Typhi	AgST-004	$1.00 \cdot 10^{9}$ CFU/mL	Negative
Salmonella	Typhimurium	AgSTM-001	$2.00 \cdot 10^{9}$ CFU/mL	Negative
Shigella	Boydii	AgSHB-00005	$2.00 \cdot 10^{8}$ CFU/mL	Negative
Shigella	Dysenteriae	AgSHD-001	$7.80 \cdot 10^{5}$ CFU/mL	Negative
Shigella	Flexneri	AgSHF-00001	$6.00 \cdot 10^{8}$ CFU/mL	Negative
Shigella	Sonnei	AgSHS-0003	$6.70 \cdot 10^{7}$ CFU/mL	Negative
Streptococcus	Pyogenes	AgT-00002	$4.80 \cdot 10^{6}$ CFU/mL	Negative
Streptococcus	Pneumococcal CWPS	AgSN-10003-01	$3.10 \cdot 10^{3}$ ng/mL	Negative
Transferrin	Human	AgTF-000001	$4.00 \cdot 10^{3}$ ng/mL	Negative
Yersinia	O:9	20200401	$8.75 \cdot 10^{7}$ CFU/mL	Negative
Yersinia	O:3	20200401	$1.25 \cdot 10^{8}$ CFU/mL	Negative

Table 2. Reference pathogen panel final concentration tested and Cross reactivity results.

Human coronavirus HKU1 has not been tested. For this reason, we cannot assume that cross-reaction with Human coronavirus HKU1 could not exit, even though the percentage identity of the nucleocapsid protein sequence of HKU1 with the nucleocapsid protein sequence of SARS-CoV-2 is about 35% that is considered as very low homology. (See point 15.6 Analytical specificity).

All the cultures and biological products of the list have been added to the samples at a high concentration and have conducted to negative values in all cases. Thus, no cross reactivity has been detected with any of the organisms analyzed.

The above results of Certest SARS-CoV-2 Test show that there is not cross reactivity with other pathogens, organism and substances that could cause respiratory infections.

15.11 Cross Reactivity Studies (with matrix)

A study of Certest SARS-CoV-2 Test was performed to determine the cross reactivity with common respiratory pathogens, other organisms and substances occasionally present in nasopharyngeal samples.

This evaluation was made in our facilities (CerTest Biotec S.L.) by a CerTest Laboratory Technician, technician 1, on 10 February 2021.

We have prepared different dilutions from the original source of each of the organism (culture or recombinant protein tested) using a matrix (negative sample for SARS-CoV-2 confirmed by qPCR technique from nasopharyngeal swab) and the sample diluent. In general, the tested concentrations (indicated in the following table) were high enough for excluding a significant cross reaction and at least 100 times higher than the normal cut-off of the current lateral flow CerTest tests.

The measurements have been repeated for the lot prepared: SC-018.

	Component	Lot	Expiry date
Test	Certest SARS-CoV-2 Test	SC-018	2022-11
Buffer	DIL10	DIL10-518	2025-12
Matrix	Nasopharyngeal sample (confirmed as negative for SARS-CoV-2 by qPCR)	NA	NA
Antigens	Culture/recombinant protein	See information on table 4	NA

Table 3. Certest SARS-CoV-2 lots used in the study.

Results of Certest SARS-CoV-2 Test cross reactivity are summarized in the following Table (Table 4).

Interpretation: Positive: Cross Reactivity; Negative: Non cross reactivity.

Microorganism	Strain/Type	Lot number	Concentration	Result
Adenovirus	Type 5 Hexon protein	AgA-00004	$1.17 \cdot 10^3$ ng/mL	Negative
Astrovirus	Capsid recombinant protein	AgAT-00005	$3.12 \cdot 10^3$ ng/mL	Negative
Calprotectin	HCT	AgHCT-00005	$5.00 \cdot 10^3$ ng/mL	Negative
Campylobacter	Jejuni	AgCA-011	$1.00 \cdot 10^6$ CFU/mL	Negative
Clostridium difficile Ag	GDH	AgGD-00004	$4.00 \cdot 10^2$ ng/mL	Negative
Clostridium difficile Tox	Purified Toxin A	TXAE-C0007	$2.00 \cdot 10^3$ ng/mL	Negative
Clostridium difficile Tox.	Purified Toxin B	AgTB-00004	$7.80 \cdot 10^3$ ng/mL	Negative
Cryptosporidium	Parvum	AgK-014	$1 \cdot 10^6$ oo/mL	Negative
Entamoeba	Recombinant protein EH29 histolytica	AgEH-00006	$1.56 \cdot 10^3$ ng/mL	Negative
Escherichia coli	O:157	AgE-00004	$1.10 \cdot 10^6$ CFU/mL	Negative
Giardia lamblia	Alfa 1 giardin	AgA1G-00007	$6.00 \cdot 10^2$ ng/mL	Negative
Giardia lamblia	CPW1	AgGP-00001	$7.80 \cdot 10^2$ ng/mL	Negative
Haemoglobin	Human	AgF-00001	$5.00 \cdot 10^3$ ng/mL	Negative
Haemoglobin	Pig	090K7609	$5.00 \cdot 10^4$ ng/mL	Negative
Haemoglobin	Bovine	10K7618	$5.00 \cdot 10^4$ ng/mL	Negative
Helicobacter pylori	Culture	AgP-011	$1.10 \cdot 10^6$ CFU/mL	Negative
Influenza A	Recombinant nucleoprotein	AgY-00008	$6.25 \cdot 10^3$ ng/mL	Negative
Influenza B	Recombinant nucleoprotein	AgYB-00006	$2.50 \cdot 10^3$ ng/mL	Negative
Lactoferrin	From human milk	AgLF-00002	$1.00 \cdot 10^4$ ng/mL	Negative
Legionella	Pneumophila (Ag)	A106FGF	$6.23 \cdot 10^3$ ng/mL	Negative
Listeria	Monocytogenes	AgLM-00006-03	$1.00 \cdot 10^6$ CFU/mL	Negative
Norovirus GI	GI.1	14012301	$4.45 \cdot 10^3$ ng/mL	Negative
Norovirus GII	GII.4	AgNP-00004	$5.20 \cdot 10^1$ ng/mL	Negative

Microorganism	Strain/Type	Lot number	Concentration	Result
Rotavirus	VP6	AgRP-00004	$1.56 \cdot 10^6$ ng/mL	Negative
Respiratory Syncytial Virus	RSV3MHis	AgRVP-00007	$1.00 \cdot 10^4$ ng/mL	Negative
Salmonella	Enteritidis	AgSE-00007	$1.00 \cdot 10^6$ CFU/mL	Negative
Salmonella	Paratyphi	AgSPA-002	$1.00 \cdot 10^6$ CFU/mL	Negative
Salmonella	Typhi	AgST-004	$1.00 \cdot 10^6$ CFU/mL	Negative
Salmonella	Typhimurium	AgSTM-001	$1.00 \cdot 10^6$ CFU/mL	Negative
Shigella	Boydii	AgSHB-00005	$1.00 \cdot 10^6$ CFU/mL	Negative
Shigella	Dysenteriae	AgSHD-002	$1.00 \cdot 10^6$ CFU/mL	Negative
Shigella	Flexneri	AgSHF-00002	$1.00 \cdot 10^6$ CFU/mL	Negative
Shigella	Sonnei	AgSHS-0004	$1.00 \cdot 10^6$ CFU/mL	Negative
Streptococcus	Pyogenes	AgT-00002	$1.00 \cdot 10^6$ CFU/mL	Negative
Streptococcus	Pneumococcal CWPS	AgSN-10003-01	$3.10 \cdot 10^3$ ng/mL	Negative
Transferrin	Human	AgTF-000002	$4.00 \cdot 10^3$ ng/mL	Negative
Yersinia	O:9	20180625	$1.00 \cdot 10^6$ CFU/mL	Negative
Yersinia	O:3	20180625	$1.00 \cdot 10^6$ CFU/mL	Negative

Table 4. Reference pathogen or substance final concentration tested and Cross reactivity results.

All the cultures and biological products of the list have been added to the samples at a high concentration and have conducted to negative values in all cases. Thus, no cross reactivity has been detected with any of the organisms analyzed.

The above results of Certest SARS-CoV-2 Test show that there is not cross reactivity with other pathogens, organism and substances that could cause respiratory infections.

The results were the same that in the experiment of cross reactivity without matrix (directly using sample extraction diluent). There is no change when using in cross reactivity a matrix (nasopharyngeal sample).

15.12 Interferences

A study of Certest SARS-CoV-2 Test was conducted to determine the existence -or not- of interferences on the test result when analysing nasopharyngeal samples for the detection of Coronavirus SARS-CoV-2 antigen.

This is an internal evaluation which was made by a CerTest Laboratory Technician, technician 1, on 1st-2nd September 2020.

The measurements have been repeated for the 3 lots prepared: SC-001, SC-002 and SC-003 (*).

Additional interferences have been evaluated later (30/11/2020) with lot number SC-011 (**) with lots SC-018, SC-019 and SC-020 (15/01/2021 and 10/02/2021) (***).

Components used (see table below):

	Component	Lot	Expiry date
Test (*)	Certest SARS-CoV-2 Test	SC-001, SC-002, SC-003	2022-08
Buffer (*)	DIL10	DIL10-354, DIL10-355, DIL10-356	2025-08
Antigens (*)	SARS-CoV-2 Culture Zeptometrix	324609	2023-07
Test (**)	Certest SARS-CoV-2 Test	SC-011	2022-10
Buffer (**)	DIL10	DIL10-406	2025-10
Test (***)	Certest SARS-CoV-2 Test	SC-018, SC-019, SC-020	2022-11
Buffer (***)	DIL10	DIL10-381, DIL10-417, DIL10-518	2025-10, 2025-10, 2025-12
Antigens (**)	SARS-CoV-2 Recombinant Nucleoprotein (NP)	CN186NP-C012	2020-09
Antigens (***)	SARS-CoV-2 Recombinant Nucleoprotein (NP)	CN186NP-C009 (AgCV-00003)	2021-06

Table 5. Certest SARS-CoV-2 Test and sample diluent lots used in this study.

For the present study, negative and positive samples have been analysed with and without the interference substances

All the positive samples analysed have been prepared by spiking them (Coronavirus SARS-CoV-2) 10x the sample diluent (buffer) of the test:

- (*) Coronavirus SARS-CoV-2 antigen: RI-4475; lot: 324609; equivalent concentration: 40.0 ng/mL (with lots SC-001, SC-002 and SC-003) (*)
- (**) Coronavirus SARS-CoV-2 antigen: RI-4409I; lot: CN186NP-C012; concentration: 40.0 ng/mL.

One initial solution has been prepared with a high enough antigen concentration for obtaining a none-strong positive result. The final concentrations selected were:

- Coronavirus SARS-CoV-2 antigen: 3.0 ng/mL (3xLoD) with SC-011 (**)
- (***) Coronavirus SARS-CoV-2 antigen: RI-4409I; lot: CN186NPC-009 (AgCV-00003); concentration: 0.7 mg/mL.

One initial solution has been prepared with a high enough antigen concentration for obtaining a none-strong positive result. The final concentrations selected were:

- Coronavirus SARS-CoV-2 antigen: 3.0 ng/mL (3xLoD) with SC-018, SC-019 and SC-020 (***)

This stock solutions will be later slightly diluted with a fixed volume of the interference substances or with the sample dilution buffer (for not interfered systems). Anyway, the final concentration after this dilution process is 90% of the original one.

15.12.1 Interfering substances Analysed

The following substances have been analysed for interferences. In all cases 10x concentrated solutions have been prepared for their subsequent 1/10 dilution, given then the final concentrations indicated in Table 4.

In the cases where drugs are studied (for exogenous interference study), the used concentrations are higher than the expected physiological values.

For endogenous interferences, we have considered concentrations 100 times higher than that of the sHOHFW-off Cut-off (concentration that differentiates patients with and without the disease) when the antigen is related with a disease.

Substance	Final concentration
Exogenous interferences	
Metronidazole (*), (***)	3.0 mg/mL
Ampicillin (*), (***)	3.0 mg/mL
Oseltamivir (*), (***)	$3.0 \cdot 10^{2}$ mg/mL
Amantadine (*), (***)	0.3 mg/mL
Ribavirin (*), (***)	3.0 mg/mL
Codeine Tos (Tosina) (*), (***)	0.2 mg/mL
Benzocaine Ang (Angileptol) (*), (***)	$3.0 \cdot 10^{-2}$ mg/mL
Cloperastine Flu (Flutox) (*), (***)	0.3 mg/mL
Carbocisteine (niston mucolítico) (*), (***)	$3.0 \cdot 10^{2}$ mg/mL

Loratadine (*), (***)	0.3 mg/mL
Dexchlorpheniramine Polaramine) (*), (***)	0.3 mg/mL
Ebastine (Ebastel) (*), (***)	3.0 mg/mL
Acetyl Salicylic (Adiro) (*), (***)	0.3 mg/mL
Ibuprofen (Espidifen) (*), (***)	0.3 mg/mL
Paracetamol Dolocatil) (*), (***)	5.0 mg/mL
Metamizole (Nolotil) (*), (***)	5.0 mg/mL
Prednisone (*), (***)	0.3 mg/mL
Omeprazole (*), (***)	$2.0 \cdot 10^{3}$ mg/mL
Loperamide hydrochloride Fortasec) (*), (***)	0.15 mg/mL
Heparin (Hibor) (*), (***)	350.0 IU/mL
Almagato (Almax) (*), (***)	3.0 mg/mL
Fosfamycin (Monurol) (*), (***)	$3.0 \cdot 10^{-3}$ mg/mL
Aceltylcysteine Fluimucil) (*), (***)	$3.0 \cdot 10^{-2}$ mg/mL
Dexketoprofen trometamol Enantyum)	0.3 mg/mL
Levofloxacin (*), (***)	$3.0 \cdot 10^{2}$ mg/mL
Ciprofloxacin (*), (***)	0.3 mg/mL
Rifampicin (Rifaldin) (*), (***)	0.3 mg/mL
Phenoxymethylpenicillin potassium (*), (***)	3.0 mg/mL
Ambroxol hydrochloride (Mucosan) (*), (***)	0.3 mg/mL
Macrogol 3350 (Movicol) (*), (***)	3.0 mg/mL
Lysine Carbocysteinate Pectox) (*), (***)	$3.0 \cdot 10^{2}$ mg/mL
Hydroxyzine dihydrochloride (*), (***)	0.3 mg/mL
Lorazepam (*), (***)	$3.0 \cdot 10^{3}$ mg/mL
Amoxicillin (*), (***)	3.0 mg/mL
Mercaptopurine (*), (***)	0.3 mg/mL
Biotine (**) (***)	100.0 µg/mL
Naso GEL (***)	0,9 mg/ml
CVS Nasal Spray (Cromolyn) (***)	4 mg/mL
Afrin (Oxymetazoline) (***)	0.05 mg/mL
CVS Nasal Drops (Phenylephrine) (***)	10 mg/mL
ZICAM (***)	0.1 mg/mL
Homeopathic (***)	DIL1/10
Sore Throat Phenol spray (***)	0.51 mg/mL
Tobramycin (***)	0.3 mg/mL
Mupirocin (***)	0.025 mg/mL
Fluticasone Propionate (***)	0.05 mg/mL
Endogenous Interferences	
Human Haemoglobin (*), (***)	5.0 µg/mL
Human Transferrin (*), (***)	0.5 µg/mL
Human Calprotectin (*), (***)	5.0 µg/mL
Human Lactoferrin (*), (***)	5.0 µg/mL
Mucine (**), (***)	5.0 mg/mL
Human blood (**), (***)	50.0 mg/mL

Table 6. Interfering Substances and concentrations analysed.

(*) these interferences were tested with SC-001, SC-002, SC003 (02/09/2020).

(**) these interferences were tested with SC-011 (30/11/2020).

(***) these interferences were tested with SC-018, SC-019 and SC-020 (15/01/2021 and 10/02/2021).

For each single measurement, the following volumes of the different solutions have been previously mixed:

- 90 µL of the positive sample stock solution (Coronavirus SARS-CoV-2 antigen), for analysing interference on positive samples, ³3RVLWLYR´ RU RI WKH negative sample stock solution (no Coronavirus SARS-CoV-2 antigen), for analysing interference on negative samples ³1HJDWLYR´
- 10 µL of the sample dilution buffer (for samples without interference agent) or of the different 10x interference agent solutions (for samples with the interference substances).

15.12.2 Results

Results of Certest SARS-CoV-2 Test interferences are summarized in the following Table 7 ³3RVLWLYR´ KDV VDPSOHV FRQWDLQLQJ Coronavirus SARS-CoV-2 antigen, ³1HJDWLYR´ refers to a sample non containing Coronavirus SARS-CoV-2 antigen.

Substance	Certest SARS-CoV-2 Test		Result
	Positive	Negative	
No substance added (reference)	+	-	OK
Exogenous Interferences			
Metronidazole (*), (***)	+	-	OK
Ampicillin (*), (***)	+	-	OK
Oseltamivir (*), (***)	+	-	OK
Amantadine (*), (***)	+	-	OK
Ribavirin (*), (***)	+	-	OK
Codeine (Tosein)	+	-	OK
Benzocaine (Angileptol) (*), (***)	+	-	OK
Cloperastine (Flutox) (*), (***)	+	-	OK
Carbocisteine (Fluimucil) (*), (***)	+	-	OK
Loratadine (*), (***)	+	-	OK
Dexchlorpheniramine (Polaramine) (*), (***)	+	-	OK
Ebastine (Ebastel) (*), (***)	+	-	OK
Acetyl Salicylic (Aditor) (*), (***)	+	-	OK
Ibuprofen (Espidifen) (*), (***)	+	-	OK
Paracetamol (Dolocatil) (*), (***)	+	-	OK
Metamizole (Nolotil) (*), (***)	+	-	OK

Prednisone (*), (***)	+	-	OK
Omeprazole (*), (***)	+	-	OK
Loperamide hydrochloride (Fortasec) (*), (***)	+	-	OK
Heparin (Hibor) (*), (***)	+	-	OK
Almagato (Almax) (*), (***)	+	-	OK
Fosfamycin (Monurol) (*), (***)	+	-	OK
Acetylcysteine (Fluimucil) (*), (***)	+	-	OK
Dexketoprofen trometamol (Enantyum) (*), (** *)	+	-	OK
Levofloxacin (*), (***)	+	-	OK
Ciprofloxacin (*), (***)	+	-	OK
Rifampicin (Rifaldin) (*), (***)	+	-	OK
Phenoxymethylpenicillin potassium (*), (***)	+	-	OK
Ambroxol hydrochloride (Mucosan) (*), (***)	+	-	OK
Macrogol 3350 (Movicol) (*), (***)	+	-	OK
Lysine Carbocysteinate (Pectox) (*), (***)	+	-	OK
Hydroxyzine dihydrochloride (*), (***)	+	-	OK
Lorazepam (*), (***)	+	-	OK
Amoxicillin (*), (***)	+	-	OK
Mercaptopurine (*), (***)	+	-	OK
Biotine (**), (***)	+	-	OK
Naso GEL (***)	+	-	OK
CVS Nasal Spray (Cromolyn) (***)	+	-	OK
Afrin (Oxymetazoline) (***)	+	-	OK
CVS Nasal Drops (Phenylephrine) (***)	+	-	OK
ZICAM (***)	+	-	OK
Homeopathic (***)	+	-	OK
Sore Throat Phenol spray (***)	+	-	OK
Tobramycin (***)	+	-	OK
Mupirocin (***)	+	-	OK
Fluticasone Propionate (***)	+	-	OK
Endogenous Interferences			
Human Haemoglobin (*), (***)	+	-	OK
Human Transferrin (*), (***)	+	-	OK
Human Calprotectin (*), (***)	+	-	OK
Human Lactoferrin (*), (***)	+	-	OK
Mucine (**), (***)	+	-	OK

Human blood (**), (***)	+	-	OK

Table 7. Interference Results.

Certest SARS-CoV-2 Test performance show no relevant interference with the now analysed products at the studied concentrations. These substances could be present in nasopharyngeal samples because of different therapies (exogenous interferences) or diseases (endogenous interferences) at concentrations lower than the analysed ones.

All the measurement performed with and without possible interfering substances with negative and positive samples have conduct to the expected result. Thus, interference (exogenous or endogenous) has not been detected with any of the analysed protocol at the analysed concentrations (always kept high enough), nor for negative neither for positive samples.

These results demonstrate the robustness of Certest SARS-CoV-2 Test.

15.13 Microbial Interferences

Microbial interferences on the test result when analysing nasopharyngeal samples for the detection of Coronavirus SARS-CoV-2 antigen.

This is an internal evaluation which was made by a CerTest Laboratory Technician, technician 1, on 9th February 2021.

The measurements have been repeated for the 3 lots prepared: SC-018, SC-019 and SC-020.

Components used (see table below):

	Component	Lot	Expiry date
Test	Certest SARS-CoV-2 Test	SC-018, SC-019, SC-020	2022-11
Buffer	DIL10	DIL10-518	2025-12
Antigens	SARS-CoV-2 Recombinant nucleoprotein (NP)	C186NP-C009 (AgCV-00003)	2021-06

Table 8. Lots used in this study.

For the present study, negative and positive samples have been analysed with and without the interference substances

All the positive samples analysed have been prepared by spiking them with Coronavirus SARS-CoV-2) 10x the sample diluent (buffer) of the test:

- Coronavirus SARS-CoV-2 antigen: RI-4409I; lot: C186NP-C009; equivalent concentration: 0.70 mg/mL.

One initial solution has been prepared with a high enough antigen concentration for obtaining a none-strong positive result. The final concentrations selected w

- Coronavirus SARS-CoV-2 antigen: 3.0 ng/mL (3xLoD)

The following samples (confirmed as positive by qPCR technique for Strep A, Adeno, Flu A, Flu B and RSV) have been analyzed for microbial interferences. The positivity of these samples is showed by the Ct value for qPCR. These samples were tested with SC-018, SC-019 and SC-020 (following IFU procedure) and results were the expected one, all of them were negative for SC-018, SC-019 and SC-020. Then these samples were tested again but previously were spiked with SARS-CoV-2 recombinant nucleoprotein obtaining a final concentration of 3xLoD of SARS-CoV-2 nucleoprotein. In this occasion the results were all of them positive as expected.

				SC-018, SC-019, SC-020	SC-018, SC-019, SC-020
No	Number of sample	Substance (microbial interference)	Ct	No SARS-CoV-2 antigen added (negative sample for SARS-CoV-2)	SARS-CoV-2 antigen added (final concentration: 3ng/mL)
1	12266	STREP A	23.7	-	+
2	12267	STREP A	22.71	-	+
3	12268	STREP A	23.35	-	+
4	9199	ADENO	24.1	-	+
5	9200	ADENO	22.8	-	+
6	17946	ADENO	19.2	-	+
7	801503	FLU A	23.47	-	+
8	801325	FLU A	22.05	-	+
9	801944	FLU A	22.87	-	+
10	803315	FLU B	32.59	-	+
11	802468	FLU B	35.61	-	+
12	802960	FLU B	25.36	-	+
13	800713	RSV	29.28	-	+
14	801505	RSV	27.78	-	+
15	802467	RSV	38.82	-	+

Table 9. Microbial interferences tested and results.

As conclusion we can see that the CerTest SARS-CoV-2 for antigen detection there is no any interaction with the microbial interferences studied in this report: STREP A, ADENO, FLU A, FLU B and RSV (respiratory pathogens).

15.14 Reproducibility with Real Samples

This assay consists on the evaluation several positive and negative samples for Coronavirus SARS-CoV-2 antigen. These samples were evaluated with three lots (SC-001, SC-002 and SC-003) and three repetitions for each sample with each lot. The samples used frozen and previously characterized by PCR Technique as positive or negative. This evaluation was performed by laboratory technician, technician 5, following the Instructions for use.

Positive Samples									
Temp: 23.4 RH: 46.7 % (EQ-1033) 07-09-2020	SC-001 DIL 10-354 (2025-08)			SC-002 DIL10-355 (2025-08)			SC-003 DIL10-356 (2025-08)		
Positive Samples	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
SW283	+	+	+	+	+	+	+	+	+
SW284	+	+	+	+	+	+	+	+	+
SW285	+	+	+	+	+	+	+	+	+
SW286	+	+	+	+	+	+	+	+	+
SW289	+	+	+	+	+	+	+	+	+

Negative Samples									
Temp: 23.4 RH: 46.7% (EQ-1033) 07-09-2020	SC-001 DIL 10-354 (2025-08)			SC-002 DIL10-355 (2025-08)			SC-003 DIL10-356 (2025-08)		
Negative Samples	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
2079019	-	-	-	-	-	-	-	-	-
2079098	-	-	-	-	-	-	-	-	-
2078892	-	-	-	-	-	-	-	-	-
2078915	-	-	-	-	-	-	-	-	-
20029564	-	-	-	-	-	-	-	-	-

The reproducibility with real samples found is as good as expected. Therefore, it can be concluded that the Certest SARS-CoV-2 Test performs correctly when analyzing samples.

15.15 Stability undiluted swabs Samples

Positive Samples in SARS-CoV-2 from two different patients were taken at the same time (nasopharyngeal swabs) and they were preserved at room temperature and tested at different period of time.

At room temperature:

Positive Samples						
Temp: 23.4, RH: 46.7 % (EQ-1033) Date sample taken: 22-10-2020	SC-002 DIL 10-355 (202508)					
Time since sample was taken	0 hours	2 hours	10 hours	16 hours	22 hours	72 hours
Patient 1	+	+	+	+	+	+
Patient 2	+	+	+	+	+	+

Our recommendations are that nasopharyngeal swab samples (in dry state) before to be diluted in the sample diluent or transport media could be stable for at least 8 hours at room temperature. Preferable take the sample and carry out the assay (test).

At 2-8ºC:

Positive Samples						
Temp: 4ºC+/-3 (EQ-1717x) Date sample taken: 22-10-2020	SC-002 DIL 10-355 (202508)					
Time since sample was taken	0 hours	2 hours	10 hours	16 hours	22 hours	72 hours
Patient 1	+	+	+	+	+	+
Patient 2	+	+	+	+	+	+

Our recommendations are that nasopharyngeal swab samples (in dry state) before to be diluted in the sample diluent or transport media could be stable for at least 8 hours at fridge Preferable take the sample and carry out the assay (test).

15.16 Stability diluted swabs Samples (real samples)

Two replicates of fifty positive samples (from nasopharyngeal swab) were diluted in several transport media (Saline Buffer, VTM and UTM) and sample diluent SARS-CoV-2. One replicated of the 15 diluted samples were preserved at room temperature and the other replicate was preserved at 2-8ºC.

These samples were evaluated after 0, 1, 2 and 6 hours after dilution in several medio and temperature conditions in order to determine how this could affect to the preservation of the samples.

At 2-8ºC:

Positive Samples						
Temp: 4ºC+/-3 (EQ-1717x) Date 09-10-2020			SC-003		DIL 10-356 (202508)	
Sample	Reference	Medium (dilution)	0 hours	1 hour	2 hours	6 hours
Positive 1	30201245	SB	++	++	++	++
Positive 2	30202719	SB	+	+	+	+
Positive 3	30207325	SB	++	++	++	++
Positive 4	30200203	SB	++	++	++	++
Positive 5	2085138	VTM	++	++	++	++
Positive 6	2085473	VTM	+	+	+	+
Positive 7	2085199	VTM	++	++	++	++
Positive 8	2084955	VTM	++	++	++	++
Positive 9	2085517	VTM	++	++	++	++
Positive 10	SW323	UTM	++	++	++	++
Positive 11	SW324*	UTM	±	±	-	-
Positive 12	SW325	UTM	+	+	+	+
Positive 13	SW326	UTM	++	++	++	++
Positive 14	SW327	UTM	+	+	+	+
Positive 15	Sample CB	DIL-10	++	++	++	++

At room temperature:

Positive Samples						
Temp: 23.1 ºC; RH: 46.7 % (EQ-1033) Date 09-10-2020			SC-003		DIL 10-356 (2025-08)	
Sample	Reference	Medium (dilution)	0 hours	1 hour	2 hours	6 hours
Positive 1	30201245	SB	++	++	++	++
Positive 2	30202719	SB	+	+	+	+
Positive 3	30207325	SB	++	++	++	++
Positive 4	30200203	SB	++	++	++	++
Positive 5	2085138	VTM	++	++	++	++
Positive 6	2085473	VTM	+	+	+	+
Positive 7	2085199	VTM	++	++	++	++
Positive 8	2084955	VTM	++	++	++	++
Positive 9	2085517	VTM	++	++	++	++
Positive 10	SW323	UTM	++	++	++	++
Positive 11	SW324*	UTM	±	-	-	-
Positive 12	SW325	UTM	+	+	+	+
Positive 13	SW326	UTM	++	++	++	++
Positive 14	SW327	UTM	+	+	+	+
Positive 15	Sample CB	DIL-10	++	++	++	++

Positive samples diluted in VTM, UTM or Saline Buffer (SB) are stable at least for 6 hours at room temperature and fridge (2-8ºC). (*) Limit samples cold be lost. The samples preserved on VTM, UTM or Saline Buffer are also stable at the frozen state, as was demonstrate by the different clinical evaluations performed on frozen samples preserved on these transport media.

15.17 Stability diluted swabs Samples (spiked samples)

Two replicates of 18 negative samples (from nasopharyngeal swab) for SARS-CoV-2 confirmed by qPCR technique were positivized adding SARS-CoV-2 culture (lot number/reference nCoV-2019 D614G(S)) until final concentration of 3 ng/mL (3xLoD) were diluted in several transport media (Saline Buffer, VTM and UTM) and sample diluent SARS-CoV-2. One replicated of the 18 diluted samples were preserved at room temperature and the other replicates were preserved at 2-8ºC.

These samples were evaluated after 0, 1, 2 and 6 hours after dilution in several medio and

temperature conditions in order to determine how this could affect the preservation of the samples.

At 2-8ºC:

Positive Samples						
Temp: 4ºC+/-3 (EQ-1717x) Date 10-02-2021			SC-018		DIL 10-381 (2025-10)	
Sample	Reference	Medium (dilution)	0 hours	1 hour	2 hours	6 hours
1	Spiked sample (3xLoD)	SB	+	+	+	+
2	Spiked sample (3xLoD)	SB	+	+	+	+
3	Spiked sample (3xLoD)	SB	+	+	+	+
4	Spiked sample (3xLoD)	SB	+	+	+	+
5	Spiked sample (3xLoD)	VTM	+	+	+	+
6	Spiked sample (3xLoD)	VTM	+	+	+	+
7	Spiked sample (3xLoD)	VTM	+	+	+	+
8	Spiked sample (3xLoD)	VTM	+	+	+	+
9	Spiked sample (3xLoD)	VTM	+	+	+	+
10	Spiked sample (3xLoD)	UTM	+	+	+	+
11	Spiked sample (3xLoD)	UTM	±	±	-	-
12	Spiked sample (3xLoD)	UTM	+	+	+	+
13	Spiked sample (3xLoD)	UTM	+	+	+	+
14	Spiked sample (3xLoD)	UTM	+	+	+	+
15	Spiked sample (3xLoD)	DIL-10	+	+	+	+
16	Spiked sample (3xLoD)	DIL-10	+	+	+	+
17	Spiked sample (3xLoD)	DIL-10	+	+	+	+
18	Spiked sample (3xLoD)	DIL-10	+	+	+	+

At room temperature:

Positive Samples Temp: 23.1 ºC; RH: 46.7 % (EQ-1033) Date 10-02-2021			SC-018		DIL 10-381 (2025-10)	
Sample	Reference	Medium (dilution)	0 hours	1 hour	2 hours	6 hours
1	Spiked sample (3xLoD)	SB	+	+	+	+
2	Spiked sample (3xLoD)	SB	+	+	+	+
3	Spiked sample (3xLoD)	SB	+	+	+	+
4	Spiked sample (3xLoD)	SB	+	+	+	+
5	Spiked sample (3xLoD)	VTM	+	+	+	+
6	Spiked sample (3xLoD)	VTM	+	+	+	+
7	Spiked sample (3xLoD)	VTM	+	+	+	+
8	Spiked sample (3xLoD)	VTM	+	+	+	+
9	Spiked sample (3xLoD)	VTM	+	+	+	+
10	Spiked sample (3xLoD)	UTM	+	+	+	+
11	Spiked sample (3xLoD)	UTM	±	-	-	-
12	Spiked sample (3xLoD)	UTM	+	+	+	+
13	Spiked sample (3xLoD)	UTM	+	+	+	+
14	Spiked sample (3xLoD)	UTM	+	+	+	+
15	Spiked sample (3xLoD)	DIL-10	+	+	+	+
16	Spiked sample (3xLoD)	DIL-10	+	+	+	+
17	Spiked sample (3xLoD)	DIL-10	+	+	+	+
18	Spiked sample (3xLoD)	DIL-10	+	+	+	+

Positive samples (spiked samples) diluted in VTM, UTM or Saline Buffer (SB) are stable at least for 6 hours at room temperature and fridge (2-8ºC). (*) Limit samples cold be lost. The samples preserved on VTM, UTM or Saline Buffer are also stable at the frozen state, as was demonstrate by the different clinical evaluations performed on frozen samples preserved on these transport media.

15.18 Comparison of different test formats

Different formats (simple card, combo card -double, triple and quadruple cassettes-, blister or midstream casing) of the same products are manufactured by CerTest.

By internal assays, we have confirmed that the general capability of our immunochromatographic Tests is not affected by the different formats used. (See report: Functionality study for different formats and molds for IC product).

The assays have been carried out with the product that is considered the most representative of CerTest (H. pylori), the best-selling, to verify if its sensitivity is affected according to these different considerations.

In this study, the evaluation of the possible variations between the combinations of base-cover-window, within the same provider for the same format, was also taken into account and it was verified whether for all our providers of each format you could find differences in the functionality of the product.

From the results obtained, after the variation of formats and moulds, with their corresponding variations by supplier, we can confirm that there are no significant differences between strip formats and positions to place the product strip. Therefore, it is considered that the sensitivity obtained for the different formats of the product will be equivalent to the others.

15.19 Inactivation Capability of the Sample Dilution buffer

The virus inactivation capability of the Sample Dilution Buffer provided with the CerTest SARS-CoV-2 Ag Rapid test has been analysed By Dr. Miguel Chillon of the ICREA / Universidad Autonoma de Barcelona.

The inactivation virus capability of sample dilution buffer of the test has been analysed after exposition time between 0 and 60 minutes, conducting to the following inactivation percentages:

Time (minutes)	Ct Threated sample	Relative activity	Reduction of activity (%)
0	20.8735	1.000	0
1	29.042	0.003206	99.6794
2	32.2635	0.00036	99.9640

5	33.142	0.000194	99.9806
10	33.96	0.000106	99.9894
15	34.845	0.000056	99.9944
20	37.125	0.000012	99.9988
60	36.87	0.000014	99.9986
C-	37.533	0.000009	99.9991

From these results it can be concluded that the sample dilution buffer inactivates the virus at the following levels:

- More than 99.6% in 1 minute
- More than 99.9% in 2 minutes
- More than 99.98% in 10 minutes
- More than 99.998% in 60 minutes

Thus, it can be confirmed that most of the virus infectivity disappears after dilution in the sample buffer in few minutes, helping to a reduction of the danger when manipulating COVID-19 clinical samples.

15.20 SARS-CoV-2 New Variants

A theoretical analysis of the different and main SARS-CoV-2 variants was carried out and the results are showed below.

These results are applied to all the rapid test manufactured by CerTest for antigen SARS-CoV-2 detection (combo, triple or quad format). These products detect SARS-CoV-2 nucleoprotein N.

The N protein has 419 amino acids. CerTest SARS-CoV-2 test detects the C-terminal end of this protein (specifically AA247-364).

With this theoretical assay we can conclude:

SARS-CoV-2 VUI 202012/01 (Variant under investigation, year 2020, month 12, variant 01) founded in the United Kingdom (UK).

This strain is a mutation mainly in spike proteins (S) but some mutations in zone N have been found.

Mutations in N protein: Position 3 (D3L) and position 235 (S235F).

These mutations are outside of our detection zone of the N protein: (AA247-364).

Conclusions: There should be no detection problems.

501Y.V2 (South Africa)

Mutations in N protein: Position 205 (T205I).

These mutations are outside of our detection zone of the N protein: (AA247-364).

Conclusions: There should be no detection problems.

GR/484K.V2 (B.1.1.28) (Brazilian)

Mutations in N protein: Position 80 (P80R), position 203 (R203K) and position 204 (G204R).

These mutations are outside of our detection zone of the N protein: (AA247-364).

Conclusions: There should be no detection problems.

GH/452R.V1 (B.1.429)

Mutations in N protein: Position 205 (T205I).

These mutations are outside of our detection zone of the N protein: (AA247-364).

Conclusions: There should be no detection problems.

These are our preliminary conclusions considering the mutations in several SARS-CoV-2 variants studied in this report. These results will be confirmed with real or spiked samples soon.

16 TEST EVALUATIONS

The results of the participations in several evaluations are included as a quality assurance procedure and to monitor the validity of the functionality test for Certest SARS-CoV-2 Test. These evaluations also allow us to study the bias and accuracy of the results of our test.

16.1 Clinical Evaluations

The objective of these evaluations was to test the functionality of the Certest SARS-CoV-2 Test, regarding its sensitivity and specificity under *in vitro* conditions.

16.1.1 Evaluation of the Certest SARS-CoV-2 Test vs another IC Rapid Test from one important competitor

Date	From 31/08/2020 to 4/09/2020
Location	Internal evaluation made in CerTest Biotec Laboratories
Samples	Swab nasopharyngeal samples, from two different providers, diluted in transport buffers for q-PCR analysis (UTM/VTM/SB).
Materials and Methods	Parallel evaluation of Certest SARS-CoV-2 Test and another IC tests Discrepant samples: RT-PCR (Gold-Standard).
Responsible Person	Manuel Villacampa Ruiz (CerTest Biotec)
Laboratory Technicians	Technician 5
Report Document with data and explanations	See document: SARS-CoV-2 (SC) vs Evaluation criteria (SD Bios-qPCR)-SC8.02 en rev01.2020v1 F-507 rev00 Clinical Evaluation raw data SARS-CoV-2 (SC) vs Ev. criteria-SC8.02

	TP	FP	FN	TN	N	Sensit. (%)	CI (95%)	Specif. (%)	CI (95%)	PPV (%)	CI (95%)	NPV (%)	CI (95%)
Certest SARS-CoV-2 Test Performance	69	1	5	47	122	93.2	84.9-97.8	97.9	88.9-99.9	98.6	92.3-100.0	90.4	79.0-96.8

16.1.2 Evaluation of the Certest SARS-CoV-2 Test for the rapid detection of Coronavirus SARS-CoV-2 antigen vs q-PCR.

Date	From 28/09/2020 to 29/09/2020
Location	Internal evaluation made in CerTest Biotec Laboratories
Samples	Swab nasopharyngeal samples, from a provider, diluted in transport buffer for q-PCR analysis (VTM).
Materials and Methods	The samples were evaluated in parallel by the Lateral Flow test (Certest SARS-CoV-2 Test) and by qPCR, the latter being used as Gold-standard.
Responsible Person	Manuel Villacampa Ruiz (CerTest Biotec)

Laboratory Technicians	Technician 5
Report Document with data and explanations	See document SARS-CoV-2 (SC) vs qPCR CT- SC8.04 en rev01.2020 v.1 / rev00 CerTest Clinical Evaluation raw data SARS-CoV-2 (SC)- SC8.04

	TP	FP	FN	TN	N	Sensit. (%)	CI (95%)	Specif. (%)	CI (95%)	PPV (%)	CI (95%)	NPV (%)	CI (95%)
Certest SARS-CoV-2 Test Performance	26	1	2	233	262	92.9	76.5-99.1	99.6	97.6-100.0	96.3	81.0-99.9	99.1	97.0-999

16.1.3 Evaluation of the Certest SARS- CoV-2 Test vs two other IC Rapid Test from important competitors

Date	From 13/10/2020 to 20/10/2020
Location	Internal evaluation made in CerTest Biotec Laboratories
Samples	Swab nasopharyngeal samples, from two provider, diluted in a transport buffer (VTM or UTM).
Materials and Methods	Parallel evaluation of Certest SARS-CoV-2 Test and other two IC test. Discrepant samples: RT-PCR (Gold-Standard).
Responsible Person	Manuel Villacampa Ruiz (CerTest Biotec)
Laboratory Technicians	Technician 5
Report Document with data and explanations	See document SARS-CoV-2 (SC) vs Evaluation criteria (Abbott-SD-qPCR) CT-SC8.0 rev01.2020v1 F-507 Internal Evaluation SARS2 Ag CerTest vs Abbott & SD Biosensor SC8.07 en rev01.2020v1

	TP	FP	FN	TN	N	Sensit. (%)	CI (95%)	Specif. (%)	CI (95%)	PPV (%)	CI (95%)	NPV (%)	CI (95%)
Certest SARS-CoV-2 Test Performance	49	0	3	48	100	94.2	84.1-98.8	100.0	92.6-100.0	100.0	92.7-100.0	94.1	83.8-98.8

16.1.4 External Evaluation of the Certest SARS-CoV-2 Test for the rapid detection of Coronavirus SARS-CoV-2 antigen vs q-PCR.

Date	From 19/10/2020 to 27/10/2020
Location	External evaluation made in Hospital Universitario Príncipe de Asturias (Madrid, Spain)
Samples	Swab nasopharyngeal samples, collected from patients at the Hospital Universitario Príncipe de Asturias, diluted in 3mL of UTM.
Materials and Methods	The samples were evaluated in parallel by the Lateral Flow test (Certest SARS-CoV-2 Te and by qPCR, the latter being used as Gold-standard.
Responsible People	Dr. Felipe Pérez García (Microbiology and Parasitology F.E.A, Hospital Universitario Príncipe de Asturias) Dr. Juan Cuadros (Director of Clinical Microbiology Service at Hospital Universitario Príncipe de Asturias and Associated Professor of Medicin
Report Document with data and explanations	See document: SARS-CoV-2 (SC) vs qPCR CT- SC8.12 en rev00.2020 v.00 F-507 rev00 CerTest Clinical Evaluation raw data SARS-CoV-2 (SC)- SC8.11

Certest SARS-CoV-2 Test Performance for Ct "25	TP	FP	FN	TN	N	Sensit. (%)	CI (95%)	Specif. (%)	CI (95%)	PPV (%)	CI (95%)	NPV (%)	CI (95%)
	79	0	5	150	234	94.0	86.7-98.0	100.0	97.6-100.0	100.0	95.4-100.0	96.8	92.6-98.9

16.1.5 External Evaluation of the Certest SARS-CoV-2 Test for the rapid detection of Coronavirus SARS-CoV-2 antigen vs q-PCR.

Date	From 18/10/2020 to 27/11/2020
Location	External evaluation made in Laboratoire de Virologie CHU Amiens-Picardie (Amiens, Fran
Samples	Swab nasopharyngeal samples diluted in transport buffers for qPCR analysis.
Materials and Methods	Parallel evaluation of Certest SARS-CoV-2 Test vs qPCR, the latter being used as Gold-standard. (Gold-Standard).
Responsible Person	Etienne Brochot (Laboratoire de Virrologie CHU Amiens-Picardie)
Laboratory Technicians	Technician working in COVID-19 molecular detection
Report Document with data and explanations	See documents: SARS-CoV-2 (SC) vs qPCR CT-SC8.12 en rev00.2020 v.00 F-507 rev00 Clinical Evaluation raw data SARS-CoV-2 (SC) vs IL criteria-SC8.12

Certest SARS-CoV-2 Test Performance		TP	FP	FN	TN	N	Sensit. (%)	CI (95%)	Specif. (%)	CI (95%)	PPV (%)	CI (95%)	NPV (%)	CI (95%)
	Ct ≤ 20	56	0	0	62	118	100.0	93.6-100.0	100.0	94.2-100.0	100.0	93.6-100.0	100.0	94.2-100
	Ct ≤ 25	88	0	8	62	158	91.7	84.2-96.3	100.0	94.2-100	100.0	95.9-100.0	88.6	78.7-94.9

16.1.6 External and internal Evaluation of the Certest SARS-CoV-2 Test for the rapid detection of Coronavirus SARS-CoV-2 antigen vs q-PCR (multi location).

Date	From 10/2020 to 12/2020
Location	External evaluation made in Hospital Universitario Príncipe de Asturias (Madrid, Spain) and internal made in CerTest facilities.
Samples	Swab nasopharyngeal samples, collected from patients at the Hospital Universitario Príncipe de Asturias diluted in 3mL of UTM. Swab nasopharyngeal samples, from related people with Certest company.
Materials and Methods	The samples were evaluated in parallel by the Lateral Flow test (Certest SARS-CoV-2 Test) and by qPCR, the latter being used as Gold-standard.
Responsible People	Dr. Felipe Pérez García (Microbiology and Parasitology F.E.A, Hospital Universitario Príncipe de Asturias) Dr. Juan Cuadros (Director of Clinical Microbiology Service at Hospital Universitario Príncipe de Asturias and Associated Professor of Medicin) Manuel Villacampa Ruiz (CerTest Biotec)
Report Document with data and explanations	See document: SARS-CoV-2 (SC) vs qPCR CT- SC8.17 en rev00.2021 F-507 rev00 CerTest Clinical Evaluation raw data SARS-CoV-2 (SC)- SC8.17

Certest SARS-CoV-2 Test Performance	TP	FP	FN	TN	N	Sensit. (%)	CI (95%)	Specif. (%)	CI (95%)	PPV (%)	CI (95%)	NPV (%)	CI (95%)
all Ct	150	2	24	618	768	84.0	77.1-89.5	99.7	98.8-100.0	98.4	94.5-99.8	96.3	94.5-97.6

As it can be seen in the previous tables, the global numbers are:

- Sensitivity: the mean values range between 92.9% and 100.0%
- Specificity: the mean values range between 97.9% and 100.0%
- PPV: the mean values range between 96.3% and 100.0%
- NPV: the mean values range between 98.5% and 99.1%.

17 HISTORICAL RESULTS

17.1 Analytical Sensitivities of the lots

In this section, the analytical sensitivities registered during the official Quality Control of each produced lot are compared. The measurements have been performed by diluting Coronavirus SARS-CoV-2 antigen in the sample dilution buffer; for each lot of strips, the buffer and antigen lots employed are also indicated.

This is a new product so, for the moment, the results are only for three lots.

Antigens employed in the sensitivity evaluation are the batch: C186NP-C0124-ESC-001; SC-002; SC-003.

Coronavirus SARS-CoV-2 antigen			Dil1	Dil2	Dil3	Dil4	Dil5	Dil6	Dil7	Dil8	Dil9	B	Sens. (ng/mL)
Lot	Exp. Date	DIL10	32.00	16.00	8.00	4.00	2.00	1.00	0.50	0.25	0.13	0.00	Results
SC-001	2022-08	351	+	+	+	+	+	±	-	-	-	-	1.00
SC-002	2022-08	351	+	+	+	+	+	±	-	-	-	-	1.00
SC-003	2022-08	351	+	+	+	+	+	±	-	-	-	-	1.00

As it can be seen in the previous table, the sensitivity of the last three lots is the same, being the typical value 1.00 ng/mL for Coronavirus SARS-CoV-2 nucleoprotein / $1 \cdot 10^3$ $TCID_{50}$/mL of 2019 n-CoV/USA-WA1/2020 (of the stated lots).

18 FINAL REPORT

18.1 Discussion

All the results shown in the previous sections can be summarized with:

- The Certest SARS-CoV-2 Test is valid for the diagnosis of Coronavirus SARS-CoV-2 (COVID-19) infection.
- The typical analytical sensitivity of the test is 1.00 ng/mL of Coronavirus SARS-CoV-2 and the acceptance range is 0.50 to 4.00 ng/mL of Coronavirus SARS-CoV-2 antigen
- The intra-lot reproducibility (or repeatability) is high (into the order of ±1 two-fold dilution) and into the experimental error.
- The inter-lot reproducibility is high (into the order of ±1 two-fold dilution) and into the experimental error.
- The inter-operator reproducibility is high (into the order of ±1 two-fold dilution) and into the experimental error.
- The inter-day reproducibility is high (into the order of ±1 two-fold dilution) and into the experimental error.
- The test resists the accelerated ageing process (5 days at 45ºC) without a significant modification of its performance.
- This a New launch and the Stability Assay Real Ageing will be completed until 25 months (0, 3, 6, 12,18, 25 months). In the meantime, the accelerated ageing study corroborates the good test stability.
- The Test clinical Sensitivity mean value for Coronavirus SARS-CoV-2 antigen is 94.2%, when compared to other rapid test, and 94.0% when compared to q-PCR results. Regarding the clinical Specificity mean value is 100.0%, according to current clinical evaluations.

18.2 Conclusions

From the results previously shown and summarized it can be concluded:

- The Certest SARS-CoV-2 Test performance shows high analytical sensibility, being it equal to 1.0 ng/mL of Coronavirus SARS-CoV-2 nucleoprotein antigen, being the acceptance range: 0.5 to 4.0 ng/mL of Coronavirus SARS-CoV-2 nucleoprotein antigen.
- The Certest SARS-CoV-2 Test intra-lot reproducibility (or repeatability) and inter-lot, inter-

operator, inter-day and inter-lot/sample reproducibility is high enough in all cases.

- The Certest SARS-CoV-2 Test performs correctly when analysing real samples and conducts to sensitivities in the order of 92.9 ±100.0% and specificities in the order of 100.0%
- Lateral Flow Technology reaches high enough sensitivity and specificity for being employed for Coronavirus SARS-CoV-2 infection detection.

19 PERSPECTIVE

This is the current version of the performance evaluation of this Lateral Flow Rapid test product for Coronavirus SARS-CoV-2 antigen detection.

Future versions will include all new information regarding new clinical evaluations, new lots, inter-laboratory and stability studies (the full Real Time Stability' D W D «