ABACUS Diagnostica

CE IVD

GENOMERA® SARS-CoV-2 ASSAY KIT

PACKAGE INSERT

REF CDX-130-01-20
CDX-130-01-40
CDX-130-01-100

ENGLISH

CONTENTS

1 INTENDED USE AND INTENDED USER

The GenomEra® SARS-CoV-2 Assay Kit is a rapid *in vitro* diagnostic (IVD) test for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid. The assay utilizes RT-PCR to amplify SARS-CoV-2 viral RNA on the GenomEra® CDX system from respiratory swab samples collected into Copan eSwab, Universal Transport Medium (UTM) or saline from patients who meet coronavirus disease 2019 (COVID-19) clinical and/or epidemiological testing criteria.

GenomEra® SARS-CoV-2 Assay Kit is intended to aid in the diagnosis of SARS-CoV-2 infections in humans when used in conjunction with clinical evaluation, laboratory findings, and epidemiological information. Positive results indicate the presence of SARS-CoV-2 RNA in the sample. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out (co-)infections from bacteria or other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The intended place of use is a laboratory not necessarily having a specialist in molecular biology but rather personnel who routinely perform assays and analyses in the field of *e.g.* microbiology, virology, or clinical diagnostics. GenomEra® SARS-CoV-2 test is intended to be used in a laboratory environment by laboratory personnel, *e.g.* laboratory technicians accustomed to normal laboratory methods such as pipetting, vortex mixing and using heating blocks.

2 SUMMARY AND EXPLANATION

SARS-CoV-2 belongs to the family *Coronaviridae* within genus Betacoronaviruses. Members of the family are enveloped, positive-sense single-stranded RNA (+ssRNA) viruses of zoonotic origin.[1] SARS-CoV-2 is closely related to the members of a viral species termed severe acute respiratory syndrome related CoV (SARSr-CoV), a species defined by the agent of the 2002/03 outbreak of SARS in humans[2,3]. The species also comprises a large number of viruses mostly detected in rhinolophid bats in Asia and Europe.[4] SARS-CoV-2 is the cause of the ongoing pandemic of COVID-19 that has been designated a Public Health Emergency of International Concern by the World Health Organization (WHO).[5,6]

Human to human transmission occurs primarily via respiratory droplets from coughs and sneezes within a range of about 2 metres.[7] Indirect contact via contaminated surfaces is another possible cause of infection.[8] Preliminary research indicates that the virus may remain viable on plastic and steel for up to three days, but does not survive on cardboard for more than one day or on copper for more than four hours,[9] and is inactivated by soap.[10] Viral RNA has also been found in stool samples from infected people.[11] COVID-19 is a respiratory disease and most infected people will develop mild to moderate symptoms of fever, cough, tiredness and shortness of breath, and recover without requiring special treatment. People who have underlying medical conditions and those over 60 years old have a higher risk of developing severe disease and death.[12]

Although rapid tests are needed to accelerate clinical decision-making and to take some of the workload off centralized test laboratories, RT-PCR-based assays are the standard approach for COVID-19 screening and will remain as such especially for the acute phase diagnostics. RT-PCR is also recommended by WHO[16].

3 PRINCIPLE OF THE PROCEDURE

GenomEra® CDX is a molecular diagnostics analyzer consisting of an integrated thermal cycler and a time-resolved fluorometer.[13] The Instrument is operated via the GenomEra® CDX Software.

The Instrument is used to run analyte-specific, ready-to-use GenomEra® Test Chips that have been developed for the detection of specific nucleic acid sequence(s) in different direct clinical or cultured sample matrices. All reagents required for performing the homogeneous amplification and detection steps are readily contained in dry form in the Test Chips.

The GenomEra® SARS-CoV-2 Assay Kit utilizes real-time RT-PCR and hydrolysis probes to detect unique sequence regions of SARS-CoV-2 envelope (E) and RNA-dependent RNA polymerase (RdRP) protein genes. Oligonucleotides used in the assay are presented in Table 1.

Table 1. Oligonucleotides used in GenomEra SARS-CoV-2 Assay Kit.

Envp-FWD	CATCCGGAGTTGTTAATCCAGT
Envp-REV	ACAAAGGCACGCTAGTAGTC
Envp-P	*Red615*-CGTCGGTTCATCATAAATTG-*MGB-EDQ*
RdRP-FWD	GTCACGGCCAATGTTAATGC
RdRP-REV	TAAATTGCGGACATACTTATCGG
RdRP-P	*FAM*-CTACTGATGGTAACAAA-*MGB-EDQ*

Red615, sulforhodamine 101 acid chloride; FAM, 6-carboxyfluorescein; MGB, Minor Groove Binder; EDQ, Eclipse® Dark Quencher

The assay also contains a sample processing control which is included in the GenomEra® SPC Tube. The control ensures that the sample preparation is performed according to instructions and functions as an amplification control to monitor assay inhibition. The control contains a small amount of MS2 bacteriophage which mimics the analyte viruses and contains a specific RNA sequence which is detected during the assay run. In the beginning of the automated assay run, the Test Chips are irreversibly sealed in the Instrument to minimize the risk of cross contamination. The target sequences are amplified and detected if present. The assay sequence takes approximately 70 minutes and ends with the reporting of results.

4 MATERIALS PROVIDED

The GenomEra® SARS-CoV-2 assay kit contains:

- 20/40/100x GenomEra® SARS-CoV-2 test chips
- 20/40/100x GenomEra® 1ml Buffer Ampoule
- 20/40/100x GenomEra® SPC Tubes
- Re-usable chip holder

5 MATERIALS REQUIRED BUT NOT PROVIDED

- Transport media and swabs for naso-/oropharyngeal specimens *e.g.*:
  - Copan Catalog No. 482CE. Conical tube filled with 1mL eSwab™ Liquid Amies medium packaged with one flexible minitip Nylon® flocked swab, sterile
  - Copan Catalog No. 360C. Conical tube filled with 1mL UTM™ medium packaged with one flexible minitip FLOQSwab™, sterile
  - Copan Catalog No. 305C. Conical tube filled with 3mL UTM™ medium packaged with one flexible minitip FLOQSwab™, sterile
- Thermal Block for 2 mL conical bottom tubes Biosan Bio TDB-100 **Do not use other thermal blocks!**
- GenomEra® CDX System (Instrument and Software), Abacus Diagnostica, Order No. CDX-10-020
- Vortex, *e.g.* Scientific Industries, Inc. Vortex Genie 2, Catalog No. G560E
- Micropipette (middle-range including 35 µL), *e.g.* Sartorius mLine pipette 10–100 µL, Catalog No. 725050
- Sterile filter-blocked tips, *e.g.* Thermo Fisher Scientific ART-100, Catalog No. 2065
- Disposable gloves, powderless
- GenomEra® CDX software version 1.3.25 or above
- Optical filter package for 5-plex update, Abacus Diagnostica, Order No. CDX-10-039
  - Note! Already installed in GenomEra CDX instruments shipped in 2020 and later.

6 WARNINGS AND PRECAUTIONS

- Do not run other than GenomEra® viral tests within the same assay run. Other assays are not compatible.
- Wear protective clothing and disposable gloves while handling the kit and the samples.
- Handle the infectious samples in accordance with safe microbiological laboratory procedures. Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Do not use the kit after the expiration date.
- Do not use the Test Chips if their foil pouch is torn upon arrival, desiccant is not present or is broken inside the chip pouch, dried reagents are detached, or the barcode or label is missing.
- Do not use the GenomEra® 1 ml Buffer Ampoules if the liquid is not clear, the tubes have leaked, or the caps are broken or open upon arrival.
- Do not use the GenomEra® SPC Tubes if the caps are broken or open upon arrival.
- Reagents are not interchangeable between lots.
- Do not remove the desiccant from opened Test Chip pouches.
- To avoid cross-contamination by amplification products, never open or pierce used Test Chips.
- The dried reagents start to dissolve as soon as the samples have been inserted to the Test Chips. The assay run should be started immediately (within 3 minutes) from the addition of the sample.
- Do not use calcium alginate swabs, as they may contain substances that inhibit PCR testing.
- Sigma Virocult transport medium (Medical Wire & Equipment, England) is incompatible and should not be used.
- All transport media containing denaturing agents such as guanidine-based reagents are incompatible and should not be used.
- Other than validated sample types or transport media can have adverse effects on assay performance and should not be used.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Amplification ratio is not to be used for evaluating sample viral load
- The reported Ct values do not solely determine the viral load in sample and are thus only estimates of the viral load.

7 STORAGE, STABILITY AND HANDLING

The storage temperature of the kit is from +2 to +8 °C. See the package for the expiration date. Store opened pouches closed at temperature from +2 to +8 °C up to 14 days. Do not remove the desiccant from the pouch.

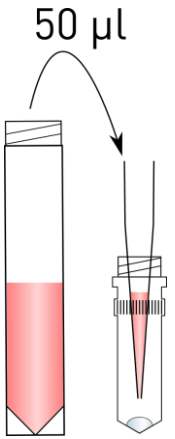
8 DIRECTIONS FOR USE

8.1 Specimen collection and preparation

The samples used with the assay comprise of respiratory swab samples which have been collected into compatible transport media. Mix the specimen received in sample collection tube by vortexing for 5 seconds.

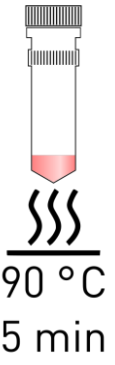
Note: 3 ml UTM tubes can be used but 1 ml tubes are more recommendable to ensure optimal sensitivity.

Sample preparation:

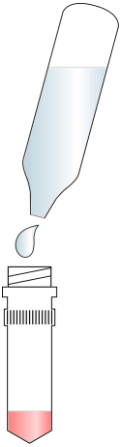


1. Vortex the original sample for 5 seconds. Pipette 50 µl of the specimen into the bottom of the GenomEra® SPC tube to rehydrate the dried sample processing control.

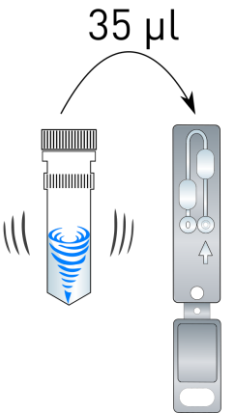
Avoid pipetting mucus and solid secretions.



2. Close the sample tube and heat the sample for 5 minutes at 90 °C.



3. Empty the contents of the 1 ml Buffer Ampoule into the sample tube.



4. Vortex the sample for 5 seconds, pipette 35 µl of the sample to the Test Chip and start the assay.

Avoid pipetting precipitates which may form during the heating step.

Note: Prepare all specimens for the assay run before pipetting to Test Chips. To run the assay, continue to section 8.3.

8.2 Storage of specimens

Specimens should be transported on ice and stored at +2 to +8 °C. For long term storage specimens may be frozen at -70 °C or colder.

Copan UTM™ specimens: Store in refrigerator (+2 °C to +8 °C) up to 96 hours.

Copan eSwab™ specimens: Store in refrigerator (+2 °C to +8 °C) up to 96 hours.

Saline specimens: Store in refrigerator (+2 °C to +8 °C) up to 96 hours.

Pretreated specimens: Store in refrigerator (+2 °C to +8 °C) up to 3 hours.

Note: Prior analysis, store specimens in original sample collection tube.

8.3 Test procedure

Prepare all samples for the same assay run as instructed above before starting any of the steps below. Ensure that the Instrument is in standby (the indicator LED light on the top cover of the Instrument is green and the 'Run assays…' button is visible). Also, ensure that the lot code of the kit has been downloaded to the Software. Please see further instructions in the GenomEra® CDX User manual.

The arrow on the chip indicates the correct opening. The dried reagents can be seen within the reaction chamber. The second chamber is an expansion chamber for accommodating vapour pressure created during the PCR heating-cooling cycles and is not to be filled.

1. Pipette 35 µL of the prepared sample per Test Chip. Use one chip per sample. If necessary, use Dummy chips to fill in empty positions in the chip holder.
2. Close the lids of the Test Chips and the lid of the chip holder.
3. Start the assay run with 4 chips by pressing the 'Run assays…' button in the GenomEra® CDX Software.

Important notes:

Carefully avoid forming bubbles while pipetting. Insert the filled pipette tip into the sample opening of the Test Chip in an upright position and ensure that the tip is firmly in place in the opening. Use a pipette that ranges below the target volume of 35 µL, *e.g.* down to 10 µL. If the pipette still easily produces bubbles, use the reverse pipetting technique. In case of visible bubbles in the reaction chamber, discard the chip and start again with a new one.

Note the order of the chips in the chip holder! The positions are numbered based on the direction the chips are moved within the Instrument. The numbering is shown on the chip holder. Carefully check that the naming of samples is in accordance with their position in the chip holder. Use the sample-ID sheet for tracking the samples.

Start the analysis within 3 minutes from adding the sample into the first Test Chip. The dried reagents start to dissolve immediately after contact with liquid.

9 INTERPRETATION OF RESULTS

9.1 Result interpretation by the GenomEra® CDX software

Results are automatically reported in approximately 70 minutes and are given both in written and numerical forms.

- A plus sign (+) in front of the result and a **red** colour indicate that the obtained result was **positive**, and the target sequence **was found** in the sample.
- A minus sign (-) in front of the result and a **green** colour indicate that the obtained result was **negative**, and the target sequence **was not found** in the sample.
- A question mark (?) in front of the result and an **orange** colour indicate that the obtained result was a **borderline** result. The presence of the target sequence **cannot be reliably derived**, and the result is **inconclusive**.

The possible result interpretation alternatives of the GenomEra® SARS-CoV-2 test are listed in Table 2. Please note that the GenomEra® CDX Software automatically calculates and reports the results. Cut-off and result values are explained in section 9.2.

Note! When samples close to the limit of detection are analysed, it is quite common that only one target gene (E or RdRP) is found to be positive. **However, even if only one gene is found positive, the sample should be considered positive.** There is no general rule which one of the target genes is detected more likely and this varies depending on the individual properties of clinical samples. This variation is not caused by mutations and, as per date, there are no known mutations affecting the sensitivity of GenomEra SARS-CoV-2 test.

Table 2. Result interpretation with the GenomEra® SARS-CoV-2 test.

Result interpretation	SARS-CoV-2 E	SARS-CoV-2 RdRP	SPC
+ POSITIVE	Positive result, target is detected	Positive result, target is detected	N/A [1]
- NEGATIVE	Negative result, target is NOT detected	Negative result, target is NOT detected	Detected
? BORDERLINE [2]	Borderline result, target detection is INCONCLUSIVE	Borderline result, target detection is INCONCLUSIVE	Detected
! PCR INHIBITION [3]	Target detection is negative or borderline	Target detection is negative or borderline	Not detected
! FAILED [4]	Failed	Failed	Failed
Run failed [5]	No result	No result	No result

[1] N/A, not applicable/required
[2] A borderline result will be reported if the amplification ratio (first part of the result value) is below the cut-off limit but the amplification curve is strongly non-linear.
[3] PCR inhibition result is reported if none of the target sequences nor the SPC is detected.
[4] Failed result is reported if the sample is not compatible with the system or the dry chemistry of the Test Chip fails.
[5] Run failed is not a result interpretation per se because no results are available for any of the Test Chips in the assay run. The entire run can fail only in case of a malfunction of the instrument. In such cases, please contact Technical Support.

9.2 Result value - amplification ratio and threshold cycle (Ct)

In addition to the qualitative result, a two-part quantitative *result value* is calculated for each analyte. Both parts of the result value are reported with one decimal accuracy and separated by a forward slash character (/). Example of a result value for a single analyte:

5.2/32.6

9.2.1 Amplification ratio

This is the signal ratio of the last and first measurement points of the real-time PCR. It can be used to estimate the amplification efficiency of the PCR reaction and is reported as the first part of the result value (5.2 in the example above).

Typically, depending on the fluorescence label used, a positive sample produces values from 2.0 up to 10.0, whereas negative samples are close to 1.0. The actual qualitative result (NEG/BOR/POS) is determined by the software with a combination of the amplification ratio, a lot-specific cut-off limit and a shape analysis of the amplification curve.

A cut-off value is a lot-specific limit which is determined for each amplicon during production and quality control. Cut-offs are typically between 1.2 and 2.0. However, cut-off values do not solely determine the result. If the amplification ratio is below the cut-off but the amplification curve is strongly non-linear, a borderline result is reported. An amplification ratio above the cut-off can produce a negative result if the amplification curve is strongly linear.

NOTE: Reported amplification ratio **is not to be used as a quantitative indicator** of the viral load in the sample; it merely reflects how well the target was amplified. This is always affected by a variety of unknown factors.

9.2.2 Threshold cycle

Threshold cycle is reported as the second part of the result value (32.6 in the example above) and can be used to estimate at which PCR cycle target replication starts to be detectable. Reported Ct values are always in the range of 9.0 to 44.0 and are calculated only for positive results and reported as 0.0 in all the other cases.

NOTE: Low Ct values (below 35) together with low amplification ratios (below 3.0) are an indication of slowly rising PCR curve. Such curves are occasionally encountered and are perfectly valid.

9.3 Troubleshooting guide for Borderline, PCR inhibition, Assay failed and Run failed results

Borderline result

Borderline result will be reported if the result value is below the cut-off limit but the amplification curve is strongly non-linear. In this case, the presence of the target cannot be reliably derived, and the result is inconclusive. It is likely that there were not enough viral RNA copies in the reaction. However, inhibitors in the specimen may also impair the amplification reaction resulting in a borderline result. **Please start again by preparing a new sample from the original specimen.**

PCR inhibition

None of the target sequences nor the sample processing control were detected. It is likely that there were too much of original sample in reaction or the sample contained an excessive amount of inhibitors which impaired the amplification reaction. **Please start again by preparing a new sample from the original specimen.**

Note: Unheated specimens result in PCR inhibition. Also heated specimens which have been stored for too long or against instructions can result in PCR inhibition.

Failed

The sample is not compatible with the system or the dry chemistry of the Test Chip has failed. Typically, the measured fluorescence signal has exceeded or fallen below acceptance limits. It is likely that the sample was missing, or the amount of original sample was too high. Failed result may also be encountered if the sample contains high amounts of blood. If you forgot to add the sample to the Test Chip prior to starting the analysis, take a new chip and perform a new assay. In case of Failed result, **start again by preparing a new sample from the original specimen.**

Run failed

In case of a malfunction in the GenomEra® CDX system, the entire run will be failed, and no results will be available. In such a rare case, the system will display the reason for the malfunction and instructions on how to proceed. Contact Technical Support.

Export of results to Laboratory Information System (LIS)

For the comprehensive description of the GenomEra® LIS support, please refer to the GenomEra® User Manual.

LIS reports are exported as text files in which one line of text represents one test chip. Each line contains 18 data fields, separated by a user configurable LIS-separator character (symbol | in the example below).

```
|"Sample 1"|"2020310-05"|"20180134"|"26007"|"1"|"SARS-CoV-2 E"|"SARS-CoV-2 RdRP"
|"+ Positive"|"+ Positive"|"POS"|"POS"|""|""|"3.2/31.1"|"5.0/32.4"|"+QCPASS"|"+QCPASS "|""|

|"Sample 2"|"2020310-05"|"20180134"|"26007"|"2"|"SARS-CoV-2 E"|"SARS-CoV-2 RdRP"
|"+ Negative"|"+ Negative"|"NEG"|"NEG"|""|""|"1.1/0.0"|"1.0/0.0"|""|""|""|
```

The GenomEra® SARS-CoV-2 assay specific LIS report fields are listed in Table 3.

Table 3. LIS report fields.

Field #	Field name	Field value	Field #		
1	Sample name	User added text	10	Analyte #1 short result	POS / BOR / NEG
2	Run number	YYYYMMDD-NN	11	Analyte #2 short result	POS / BOR / NEG
3	Instrument serial number	XXXXXXXX	12	Parameter #1 name	Not used (empty)
4	LOT number	26XXX	13	Parameter #2 name	Not used (empty)
5	Chip position	1 / 2 / 3 / 4	14	Analyte #1 result value	Amplification ratio/Ct
6	Analyte #1 name	SARS-CoV-2 E	15	Analyte #2 result value	Amplification ratio/Ct
7	Analyte #2 name	SARS-CoV-2 RdRP	16	Analyte #1 QC result (if available)	+/-QCPASS / +/-QCFAIL
8	Result interpretation #1 (language-dependent)	See Table 1	17	Analyte #2 QC result (if available)	+/-QCPASS / +/-QCFAIL
9	Result interpretation #2 (language-dependent)	See Table 1	18	Extra text	Not used (empty)

10 QUALITY CONTROL (QC)

10.1 Sample processing control (SPC)

SPC is designed to monitor the assay performance. SPC ensures that the sample preparation is performed according to instructions and also functions as an internal amplification control to monitor assay inhibition and reagent integrity for each specimen. PCR inhibition result is reported if none of the target sequences nor SPC have been detected.

10.2 External quality assessment

It is specifically recommended that users of this assay kit participate regularly in external quality assessment programs for molecular diagnostic testing of SARS-CoV-2. Recommended providers for such programs are *e.g.*:

LabQuality: https://www.labquality.fi/

Instand e.V.: https://www.instand-ev.de/

Note! QCMD sample matrices of respiratory panels are incompatible with the direct sample preparation method of GenomEra SARS-CoV-2 test. QCMD EQA panels will require separate nucleic acid extraction.

10.3 Control samples

The running of control samples in each assay run or at specific intervals is not required. Positive and negative controls can, however, be run anytime. Follow the laboratory policy regarding the recommended frequency of QC runs.

Control samples can be identified using +/- QC checkboxes after starting an assay run. Control samples can be included in a conventional assay run or run separately. The use of commercially available reference strains for external QC is recommended but well characterized positive and negative samples are also suitable to be used as controls.

11 PERFORMANCE CHARACTERISTICS

11.1 Clinical performance

Performance characteristics of the GenomEra® SARS-CoV-2 Assay Kit were evaluated at four institutions across Europe during the COVID-19 pandemic. One site was partially an in-house site, as GenomEra analysis for the samples from Site 4 were performed in-house. Depending on the site, study specimens consisted of fresh and frozen respiratory swab samples collected in eSwab, UTM or saline (0.9 % NaCl) from patients with signs and symptoms of respiratory infection. Reference test methods (primary and confirmatory, respectively) were; Site 1: Abbott RealTi*me* SARS-CoV-2, Seegene Allplex™ SARS-CoV-2 Assay and Cepheid Xpert Xpress SARS-CoV-2, Site 2: E gene in-house[4] RT-PCR by Corman et al. and duplex RdRP in-house[17] recommended by WHO (first validated by Pasteur Institute France), Site 3: RdRP gene in-house[4] modified from Corman et al. and Xpert® Xpress SARS-CoV-2, Site 4: Xpert® Xpress SARS-CoV-2 and duplex E and RdRP gene in-house[4] by Corman et al. Results of the clinical performance evaluation are presented in Tables 4−6.

Table 4. Overall clinical performance of GenomEra® SARS-CoV-2 Assay Kit on prospective and retrospective clinical specimens.

	n	TP	FP	TN	FN	PPA % (CI 95)	NPA % (CI 95)
Total	270	98	0	162	10[1]	**90.7** (83.6–95.5)	**100** (97.8–100)
Fresh	180	37	0	134	9	80.4 (66.1–90.6)	100 (97.3–100)
Frozen	90	61	0	28	1	98.4 (91.3–100)	100 (87.7–100)

[1] Further information on discrepant samples is presented in Table 5.

Table 5. False negative prospective samples on GenomEra from Site 3. Confirmatory method was Xpert® Xpress SARS-CoV-2 which detects the genes for nucleocapsid 2 (N2) and envelope (E) proteins.

Sample #	Reference method Ct value	Confirmatory method Ct values (E / N2)	Sample matrix
20212	36.2	34.1 / 36.5	saline
20847	35.4	32.1 / 35.1	saline
15908	34.8	31.2 / 33.7	saline
16149	37.7	- / 41.2	eSwab
16340	31.8	29.3 / 32.6	eSwab
16727	36.0	- / 39.6	eSwab
17169	37.9	- / 41.8	saline
17557	38.2	35.7 / 39.2	saline
18280	37.4	43.5 / 37.5	eSwab

Table 6. GenomEra® SARS-CoV-2 Assay Kit performance on different sample collection media.

	n	TP	FP	TN	FN	PPA % (CI 95)	NPA % (CI 95)
eSwab	134	51	0	79	4	92.7 (82.4–98.0)	100 (95.4–100)
UTM	71	16	0	52	1	94.1 (71.3–99.9)	100 (93.4–100)
saline	40	30	0	5	5	85.7 (69.7–95.2)	100 (47.8–100)
unidentified	26	1	0	24	0	100 (2.5–100)	100 (85.8–100)

A total of 272 specimens were analysed of which a valid result was obtained in 270 cases. One of the inconclusive specimens resulted in PCR inhibition, and the other in Failed result. The latter was not retested. When the inhibitory specimen was retested, a Failed result was obtained due to abnormal signal levels probably caused by interfering substances present in the sample. Therefore, status of these two samples was determined to remain inconclusive. Both samples were negative with reference method. Combined inhibition and failed rate was 0.7%. No borderline results were encountered.

11.2 Analytical sensitivity (Limit of Detection)

Analytical sensitivity of the GenomEra® SARS-CoV-2 Assay Kit was evaluated with viral genomic RNA (AmpliRun® SARS-CoV-2 RNA Control, product#: MBC137-R, Vircell, Granada, Spain) spiked into pooled negative clinical sample matrices (Copan eSwab and UTM, 0.9 % NaCl *i.e.* saline, PBS) in various concentrations. Samples were originally collected with nasopharyngeal swabs. The limit of detection (LoD) was defined as the lowest RNA concentration per sample which could be reproducibly distinguished from negative samples with 95 % confidence. Four replicates per each dilution were analysed to determine LoD estimates, which were subsequently confirmed by testing a total of 20 or 8 replicates, depending on sample matrix. Copan eSwab is the most recommended sample collection and preservation medium to be used with GenomEra SARS-CoV-2 assay, and therefore the LoD for eSwab samples was confirmed with 20 replicates. According to the recent guidance issued by European Commission[14], the determined LoDs were further confirmed by analysing half-logarithmic dilutions around LoD concentrations. Table 7 represents the analysed concentrations and the acquired results during the determination of LoD.

Table 7. Determination of LoD for different sample matrices for GenomEra SARS-CoV-2 Assay Kit.

copies/ml original specimen	copies/ml processed sample	copies/reaction	**Result** (replicates positive)
eSwab			
1143	57	2	5/8
2857	143	5	20/20
4000	200	7	7/8
5714	286	10	8/8
8571	429	15	8/8
UTM			
1143	57	2	4/8
2857	143	5	7/8
4000	200	7	7/8
saline (0.9 % NaCl)			
1143	57	2	5/8
2857	143	5	8/8
4000	200	7	5/8
PBS			
1143	57	2	2/8
2857	143	5	6/8
4000	200	7	8/8

11.3 Analytical reactivity (Inclusivity)

SARS-CoV-2 is still a novel virus, and not many aberrant sequence variants have been detected by far. A comprehensive *in silico* inclusivity study was carried out to demonstrate the strain coverage of GenomEra SARS-CoV-2 Assay Kit, but also a few available characterized viral strains were tested *in vitro* at evaluation Site 2. Dilutions of extracted viral RNA with reference Ct values approximately 30 were analysed and correctly detected for the following strains: Finland/1/20/Wuhan/China and Finland/2/20/Milano/Italia.

In silico strain coverage analyses of the oligonucleotide sequences for the E and RdRP genes were performed against all available sequences for organism SARS-CoV-2 (taxid: 2697049) in the GenBank Nucleotide Database as of July 21st 2020. Results showed that none of the target sequences contained more than a single base pair mismatch with a single primer or probe, *i.e.* the vast majority of target sequences were 100 % homological with all the primers and probes. None of the mismatches are predicted to have a negative impact on the performance of the assay.

The dual-target approach utilized in GenomEra SARS-CoV-2 test decreases the significance of potential mismatches since it is very rare that a viral strain would have generated mutations in both amplicon regions.

11.4 Analytical specificity (Exclusivity)

The analytical specificity of the GenomEra® SARS-CoV-2 Assay Kit was evaluated by testing various microorganisms which may be commonly encountered in respiratory specimens. All of the viral species and part of the bacterial species were tested at evaluation Site 2. The data was supplemented by testing a few available bacterial species in-house. Concentrations of all micro-organisms were not quantitated but the absorbances of the bacterial stocks were high enough (absorbance at 600 nm > 0.2) to ensure the detection of potential cross-reactivity. Viral concentrations were determined by PCR and presented as threshold cycles or copies/ml. For some microorganisms, specimens containing intact viruses/bacteria were not available and therefore pure nucleic acids were tested for cross-reactivity. None of the tested microorganisms caused cross-reactivity (Table 8).

Table 8. Microorganisms tested with the GenomEra® SARS-CoV-2 Assay Kit.

Organism	Concentration	Result
Coronavirus 229E	Multiple specimens with Ct values ranging from 20.64 to 27.24	Negative
Coronavirus HKU1	Multiple specimens with Ct values ranging from 17.58 to 24.95	Negative
Coronavirus NL63	Multiple specimens with Ct values ranging from 20.83 to 23.38	Negative
Coronavirus OC43	Multiple specimens with Ct values ranging from 20.19 to 26.65	Negative
SARS-CoV (RNA)	10^6 and 10^7 copies/mL	Negative
MERS-CoV (RNA)	No information of viral load	Negative
Influenza A(H1)pdm09	Two specimens with Ct values of 17.98 and 22.46	Negative
Influenza A(H3)	Two specimens with Ct values of 19.21 and 20.64	Negative
Influenza B (Victoria)	Two specimens with Ct values of 21.05 and 20.56	Negative
Influenza B (Yamagata)	Two specimens with Ct values of 21.46 and 22.35	Negative
Respiratory syncytial virus	Two specimens with Ct values of 22.02 and 22.30	Negative
Rhinovirus	Multiple specimens with Ct values ranging from 19.71 to 38.95	Negative
Adenovirus	Two specimens with Ct values of 22.02 22.19	Negative
Enterovirus D68 (RNA)	Ct value 21.33	Negative
Human parechovirus (RNA)	2×10^5 copies/mL	Negative
Metapneumovirus A1 (RNA)	No information of viral load	Negative
Metapneumovirus A2 (RNA)	No information of viral load	Negative
Parainfluenzavirus 1 (RNA)	No information of viral load	Negative
Mycoplasma pneumoniae (DNA)	Ct value 28.00	Negative
Pseudomonas aeruginosa	> 0.2 Abs600 nm	Negative
Streptococcus pneumoniae	> 0.2 Abs600 nm	Negative
Streptococcus pyogenes	> 0.2 Abs600 nm	Negative
Streptococcus salivarius	> 0.2 Abs600 nm	Negative

In addition, an *in silico* cross-reactivity analysis was performed with all GenomEra SARS-CoV-2 primer and probe sequences from the E and RdRP gene oligonucleotide sets against all available sequences of the organisms in GenBank Nucleotide database available as of July 21st 2020, which are listed in Table 2 of WHO document Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid[15].

In conclusion, *in silico* cross-reactivity analysis indicated that there are just a few oligonucleotide hits to non-target sequences with ≥ 80% homology, and all of those are non-amplifiable.

11.5 Interfering substances

Potentially interfering substances which may be present in respiratory sample matrices, were tested for effects on the performance of the GenomEra® SARS-CoV-2 Assay Kit. Potentially interfering substances were added into negative and spiked low positive (3 x LoD) sample matrices and tested as normal samples. None of the endo- or exogeneous substances had negative effects on the amplification of the target analyte nor the sample processing control. However, mucin may have negative effects on assay performance if its concentration in the sample is greater than 2.5 % (w/v).

Common laboratory disinfectants were also screened for inhibitory effects. Isopropyl alcohol was the only substance which had a discernible interfering effect by inducing a failed result due to abnormally low signal levels. All the substances and their tested concentrations are presented in Table 9.

Table 9. Potentially interfering substances tested with the GenomEra® SARS-CoV-2 Assay Kit.

Endogeneous substances	**Substance commercial trade name**	**Active ingredient**	**Tested concentration in transport medium**
Mucin	Mucin from porcine stomach Sigma-Aldrich Cat. No M1778	Purified mucin protein	2.5 % (w/v)
Blood (human)	N/A	Blood (human)	2 % (v/v)
Exogeneous substances			
Nasal corticosteroid	Beconase 50 µg/dose	Beclomethasone	5 % (v/v)
Nasal corticosteroid	Flixonase 50 µg/dose	Fluticasone	5 % (v/v)
Throat lozenge, oral anesthetic and analgesic	Bafucin	Benzocaine	5 mg/mL
Anti-viral drug	Tamiflu	Oseltamivir	7.5 mg / ml
Antibiotic, nasal ointment	Bactroban nasal 2 %	Mupirocin	10 mg/mL
Beta-adrenergic bronchodilator	Ventoline Diskus	Salbutamol	10 mg/mL
Nasal adrenergic receptor agonist	Vicks Sinex	Oxymetazoline	5 % (v/v)
Laboratory disinfectants			
Ethanol	Etax A12	Ethanol	2 % (v/v)
Hypochlorite	N/A	Sodium hypochlorite	0.01 % (v/v)
Aseptic disinfectant	Neo-Amisept	Isopropyl alcohol	1.5 % (v/v)

11.6 Reproducibility

Reproducibility of the GenomEra® SARS-CoV-2 Assay Kit was evaluated at three sites, one being an in-house site. Identical sample panels consisting of one low positive and one negative clinical sample were tested at all three sites over 5 days. Positive sample was prepared by diluting inactivated NATtrol SARS-CoV-2 (Zeptometrix, USA) stock into Copan eSwab. Negative sample was pooled nasopharyngeal matrix collected into eSwab from healthy donors. Samples included in the reproducibility study and the results of the study are presented in Table 10.

Table 10. Samples and results of the reproducibility study.

Samples	**Site 1 # of correct results per replicates**	**Site 2 # of correct results per replicates**	**In-house # of correct results per replicates**	**Total**
SARS-CoV-2 Low positive (2 x LoD)	5/5	5/5	5/5	15/15
Negative sample	5/5 (SPC positive)	5/5 (SPC positive)	5/5 (SPC positive)	15/15 (SPC positive)

11.7 Sample stability

Stability study was conducted for original untreated samples, and also for pre-treated samples. Frozen clinical samples encountered during evaluations were used as dilutions to assess stability. Table 11 summarizes the stability results for original untreated samples. Refrigerator preserved the samples for 96 hours without significant declines. In room temperature (RT), the eSwab specimen was seen to decay after 48 h of storage. If immediate testing is not possible in routine use, samples must be stored in refrigerator and tested as follows; UTM samples within 96 h, eSwab and saline samples within 48 h, as stated in section 8.2 of this document.

Table 11. Stability results for original untreated samples.

Sample	Zero-point	16 h, +4 °C	48 h, +4 °C	96 h, +4 °C	16 h, RT	48 h, RT	96 h, RT
eSwab 1:1600	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
eSwab 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	1/2 positive	1/2 positive
eSwab 1:6400	1/1 positive	n/a[1]					
UTM 1:1600	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
UTM 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
UTM 1:6400	1/1 positive	n/a[1]					
Saline 1:1600	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
Saline 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
Saline 1:6400	0/1 positive	n/a[1]					
PBS 1:1600	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
PBS 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive

[1] Time-points were not tested as the zero-point result was negative or very low positive.

The same samples were used to assess the stability of pre-treated samples. It is expected that heat-treated samples with free RNA in solution may not remain stable very long. Thus, only short storage times in refrigerator and room temperature were tested. With most of the samples, there was only a minor or no difference between 3 and 8 hours of storage. However, as also seen during the stability study for original untreated samples, eSwab specimens start to turn negative after 3 hours of storage. The data suggests that it should be relatively safe to store pre-treated samples in refrigerator and test them within 3 hours, as also stated in section 8.2 of this document. Table 12 summarizes the stability results for pre-treated samples.

Table 12. Stability results for pre-treated samples.

Sample	Zero-point	3 h, +4 °C	8 h, +4 °C	3 h, RT	8 h, RT
eSwab 1:3200	2/2 positive	2/2 positive	1/2 positive	1/2 positive	0/2 positive
UTM 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
Saline 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive
PBS 1:3200	2/2 positive	2/2 positive	2/2 positive	2/2 positive	2/2 positive

12 LIMITATIONS OF THE TEST

12.1 Inhibitors in clinical samples

The GenomEra® SARS-CoV-2 assay has been developed for the detection of viral RNA from respiratory swab specimens. The amount of inhibitors in clinical samples can be highly variable. It is important that only the instructed sample volume is used for sample preparation. When the sample preparation is performed according to the instructions, PCR inhibition is rarely encountered. However, if PCR inhibition or assay failure is detected, follow the instructions given in section 9.2.

12.2 Other limitations

It should be noted that the evolution and mutations of viral species is extremely rapid and aberrant sequence variants resulting in unspecific or hampered nucleic acid amplification may cause decrease in assay performance.

Errors in following the assay procedure may lead to false negative results.

Improper collection, storage or transport of specimens or assay kits may lead to false negative or positive results.

Negative results do not preclude the possibility of infection and should not be used as only basis for treatment or other patient management decisions. Improper collection, storage or transport of specimens or assay kits may lead to false negative or positive results.

A competent health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.

Viral RNA of the analyte may persist in samples independent of the viability of the virus. Detection of viral RNA of the analyte in samples does not imply that the corresponding virus is infectious or that they are the cause of clinical symptoms.

There is a risk of false negative values due to the presence of sequence variants in the viral target of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organism for amplification.

Positive results should be interpreted in conjunction with other laboratory findings and clinical signs or symptoms. Positive results do NOT rule out other non-target analyte causes of infection. Negative results do not exclude other types of viral or bacterial infection. Co-infections with multiple viral, bacterial or other agents are also possible.

False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may occur if inadequate numbers of virus are present in the specimen.

13 INSTRUCTIONS FOR DISPOSAL

Dispose of infectious waste in accordance with the laboratory regulations. Do not open, pierce or grind used Test Chips.

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SYMBOLS

	Manufacturer		Do not re-use
REF	Catalogue number	Σ	Contains sufficient for <n> test
LOT	Batch code		Temperature limitation
	Consult instructions for use		Use by end of this month
14 D	Shelf life, following the first opening (dd)	CE	CE-marked product
IVD	*In vitro* diagnostic medical device		

This Product incorporates technologies (Dyes, Quenchers, MGB) sublicensed by Kaneka Eurogentec S.A. exclusively for diagnostic purposes. Further information on purchasing licenses may be obtained by contacting licensing @eurogentec.com.

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