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Evaluation of GenomEra® SARS-CoV-2 Assay Kit for the Detection of SARS-CoV-2 Viral RNA from Respiratory Samples

Metropolia University of Applied Sciences

Bachelor of Laboratory Services

Laboratory Sciences

Bachelor's Thesis

13 May 2021

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Title	Evaluation of GenomEra® SARS-CoV-2 Assay Kit for the Detection of SARS-CoV-2 Viral RNA from Respiratory Samples
Number of Pages	30 pages + 1 appendix
Date	13 May 2021
Degree	Bachelor of Laboratory Services
Degree Programme	Laboratory Sciences
Instructors	Soile Blomqvist, Senior Researcher Tiina Soininen, Senior Lecturer

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the seventh identified coronavirus that causes disease in humans. SARS-CoV-2 is the causative agent of the coronavirus disease (COVID-19) pandemic.

This thesis deals with the evaluation of the clinical performance of Abacus Diagnostica's GenomEra® SARS-CoV-2 Assay Kit for the qualitative detection of SARS-CoV-2 viral RNA from respiratory tract samples. The thesis work was carried out in the virology laboratory of Finnish Institute for Health and Welfare's (THL) Expert Microbiology Unit. The purpose of the clinical evaluation was to obtain data for assessing the conformity of the assay kit for CE-IVD marking. Other study sites also participated in the clinical evaluation. However, those results are not taken into account in this thesis.

The clinical performance of the GenomEra® SARS-CoV-2 assay was evaluated through clinical sensitivity and specificity, inclusivity and cross-reactivity. Also, the reproducibility of the assay was analyzed. The results obtained with the GenomEra® SARS-CoV-2 assay were compared to results obtained with THL's validated in-house real-time RT-PCR test for SARS-CoV-2 E gene. The sample material consisted of diagnostic upper respiratory tract samples taken from suspected COVID-19 cases, as well as a panel of pathogens known to cause upper respiratory tract infections in humans. The panel included diagnostic upper respiratory tract samples and isolated RNA/DNA samples.

Due to the excellent epidemic situation in Finland in the summer of 2020, the number of COVID-19 samples analyzed fell short of what was planned. However, estimates of the clinical sensitivity and specificity of the assay were optimistic. No difference in the ability of the test to identify temporally and geographically differing SARS-CoV-2 strains was observed. Cross-reactivity was also not observed. The reproducibility testing yielded consistent results on all days.

The studies indicated GenomEra® SARS-CoV-2 assay to be a reliable test for the fast qualitative detection of SARS-CoV-2 RNA from upper respiratory tract samples. The assay was CE-IVD marked in July 2020.

Keywords	SARS-CoV-2, COVID-19, IVD, Clinical Performance

Tekijä Otsikko Sivumäärä Aika	Irmeli Iranto GenomEra® SARS-CoV-2 -testikitin evaluointi SARS-CoV-2 RNA:n osoittamiseksi hengitystienäytteistä 30 sivua + 1 liite 13.5.2021
Tutkinto	laboratorioanalyytikko (AMK)
Tutkinto-ohjelma	laboratorioanalytiikka
Ohjaajat	erikoistutkija Soile Blomqvist lehtori Tiina Soininen

Uusi koronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) on seitsemäs tunnistettu ihmiselle tautia aiheuttava koronavirus. Se aiheuttaa koronavirustautia (coronavirus disease, COVID-19), jonka maailman terveysjärjestö WHO julisti pandemiaksi 11.3.2020.

Tässä opinnäytetyössä evaluoitiin Abacus Diagnostica Oy:n GenomEra® SARS-CoV-2 RT-PCR -testin kliinistä suorituskykyä SARS-CoV-2 RNA:n kvalitatiiviseen osoittamiseen hengitystienäytteistä. Tutkimukset toteutettiin Terveyden ja hyvinvoinnin laitoksen (THL) asiantuntijamikrobiologiayksikön virologian laboratorioissa. Evaluointitutkimuksiin osallistui useampi laboratorio, ja kerättyjä tuloksia oli tarkoitus hyödyntää osana testin vaatimuksenmukaisuuden arviointia CE-IVD-merkintää varten. Tässä opinnäytetyössä käsitellään vain THL:n laboratorioissa kerättyjä tuloksia.

GenomEra® SARS-CoV-2 -testin kliinistä suorituskykyä arvioitiin kliinisen herkkyyden ja spesifisyyden, inklusiivisuuden sekä ristireagoivuuden avulla. Lisäksi selvitettiin tulosten uusittavuutta kontrollinäytteiden rinnakkaismäärityksillä eri päivinä. Testin tuloksia verrattiin referenssimenetelmällä saatuihin tuloksiin. Referenssimenetelmänä käytettiin THL:n sisäistä reaaliaikaista RT-PCR-menetelmää SARS-CoV-2 E-geenille. Näyteaineisto koostui epäillyiltä COVID-19-tapauksilta otetuista diagnostisista ylähengitystienäytteistä sekä tunnettujen ylähengitystieinfektioita aiheuttavien patogeenien paneelista, joka sisälsi diagnostisia ylähengitystienäytteitä ja eristettyjä RNA/DNA-näytteitä.

Suomessa kesällä 2020 vallinneen erinomaisen epidemiatilanteen vuoksi tutkittujen COVID-19-näytteiden määrä jäi alle suunnitellun. Kuitenkin käytetyllä näytemäärällä saadut arviot testin kliinisestä herkkyydestä ja spesifisyydestä olivat optimistisia. Inklusiivisuustulosten perusteella eroa testin kyvyssä tunnistaa ajallisesti ja maantieteellisesti eriäviä SARS-CoV-2 -kantoja ei havaittu. Myöskään ristireagoivuutta ei havaittu. Uusittavuustestauksissa saatiin yhtenevät tulokset kaikkina päivinä.

Tutkimukset osoittivat, että GenomEra® SARS-CoV-2 -testi on käyttötarkoitukseensa soveltuva. Se tunnistaa SARS-CoV-2 RNA:n luotettavasti ja nopeasti ylähengitystienäytteistä. Testi CE-IVD-merkittiin heinäkuussa 2020.

Avainsanat	SARS-CoV-2, COVID-19, IVD, kliininen suorituskyky

Contents

List of Abbreviations

CE marking	Conformité Européenne marking. Demonstrates that the product satisfies the applicable EU requirements.
CoV	Coronavirus
COVID-19	Coronavirus disease. The disease caused by severe acute respiratory syndrome coronavirus 2.
Ct value	Cycle threshold value
DNA	Deoxyribonucleic Acid
Fimea	Finnish Medicines Agency (Lääkealan turvallisuus- ja kehittämiskeskus)
IVD	*In vitro* diagnostic medical device. A medical device intended for the *in vitro* examination of specimens derived from the human body for a medical purpose.
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
THL	Finnish Institute for Health and Welfare (Terveyden ja hyvinvoinnin laitos)
WHO	The World Health Organization

1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a novel coronavirus (CoV) that causes the coronavirus disease (COVID-19) in humans and is responsible for the ongoing COVID-19 pandemic. SARS-CoV-2 is a zoonotic virus (= virus that is transmitted from animals to humans), but its zoonotic source is currently unknown. It belongs to the genera of *Betacoronavirus*es and is genetically related to a number of other CoVs isolated from bats. [1,1.]

This thesis deals with the evaluation of Abacus Diagnostica's GenomEra® SARS-CoV-2 Assay Kit's clinical performance for COVID-19 diagnostics. The test utilizes reverse transcription polymerase chain reaction (RT-PCR) to amplify and detect the genetic material of SARS-CoV-2.

Abacus Diagnostica is a Finnish company specialized in rapid molecular diagnostics. The national regulatory authority in Finland, the Finnish Medicines Agency (Fimea), granted a special approval for Abacus Diagnostica to supply the GenomEra® SARS-CoV-2 Assay Kit to Finnish markets for diagnostic use.

The project was carried out in the virology laboratory of Finnish Institute for Health and Welfare's (THL) Expert Microbiology Unit. Two other laboratories also participated in the clinical performance evaluation studies of the GenomEra® SARS-CoV-2 Assay Kit. The data gathered were used as a part to declare the conformity of the test (= requirements of Directive 98/79/EC on *in vitro* medical devices – which currently applies to COVID-19 tests – are satisfied) to allow affixing the CE-IVD (IVD= *in vitro* diagnostic medical device) marking. However, in this thesis only the data collected at THL is considered.

2 Human Coronaviruses

2.1 Classification of HCoVs

Coronaviruses are classified under the order *Nidovirales*, suborder *Cornidovirineae*, family *Coronaviridae* and subfamily *Orthocoronavirinae*. The subfamily *Orthocoronavirinae* is further divided into four genera: *Alpha*-, *Beta*-, *Gamma*-, and *Deltacoronavirus*. [2;3,2.] Coronaviruses that are known to cause infections in humans (Human Coronavirus, HCoV) belong to *Alpha*- and *Betacoronavirus*es. The seven coronaviruses known to infect humans include: HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV) and SARS-CoV-2 (table 1). [4.]

Table 1. Classification of human coronaviruses. Data gathered from Lappalainen & Julkunen (2020) [4].

Genus	Strain	Discovery	Receptor
Alphacoronavirus	HCoV-229E	1966	Aminopeptidase N
	HCoV-NL63	2004	Angiotensin converting enzyme 2 (ACE2)
Betacoronavirus	HCoV-OC43	1967	9-O-Acetylated sialic acid
	HCoV-HKU1	2005	9-O-Acetylated sialic acid
	SARS-CoV	2003	ACE2
	MERS-CoV	2012	Dipeptidyl peptidase 4
	SARS-CoV-2	2020	ACE2

HCoVs -229E, -NL63, -OC43 and -HKU1 are known as common human coronaviruses that circulate globally in the human population. None of these common HCoVs have been found to persist within an animal reservoir. In contrast, SARS-CoV and MERS-CoV are not as well adapted to persist in humans, but are instead preserved within animal reservoirs. [5.]

2.2 Structure and Replication of HCoVs

Coronaviruses are a large group of single-stranded (ss), positive-sense ribonucleic acid (RNA) viruses. With a genome consisting of approximately 27 to 32 kilobases, coronavirus possess the largest viral RNA genome described to this day. [3,3.] The

name coronavirus comes from the crown-like appearance of the spike (S) protein that coats the lipid envelope of the virus [4]. Main structure of the coronavirus is illustrated in figure 1.

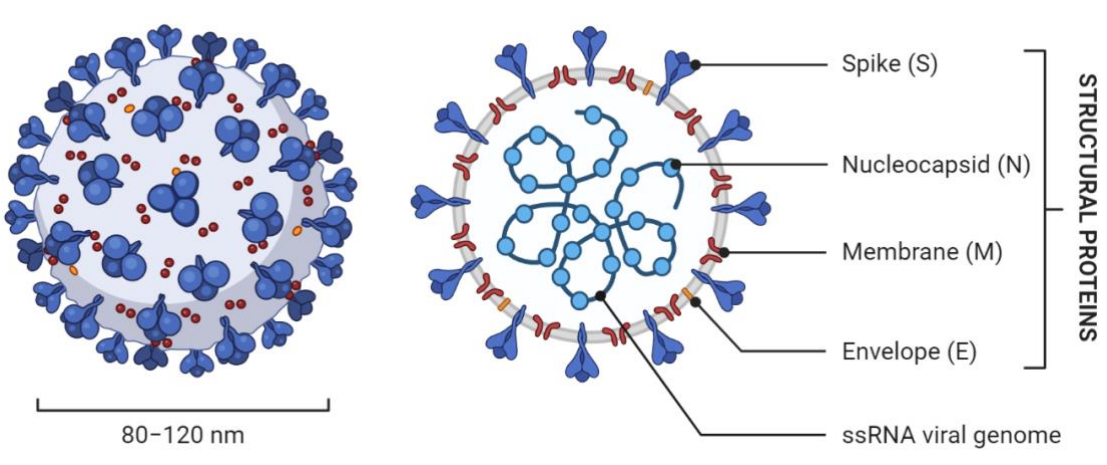

Figure 1. Main structure of human coronavirus. The genetic material contained in the core is surrounded by a lipid envelope with protein spikes on the surface. Figure adapted from “Coronavirus Structure and Protein Visualization”, by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates.

Three major structural proteins of human coronaviruses include: the spike protein (S), the membrane protein (M) and the nucleocapsid protein (N). There is also a minor envelope protein (E), and in some coronaviruses an additional envelope protein, hemagglutinin-esterase (HE). [3,2;6,437–438.]

The S protein is a transmembrane protein with a molecular weight of 128–160 kilodaltons (kDa). It is responsible for the attachment of a virus particle to a host cell. [3,2.] The S protein has two functional domains, S1 (bulb) and S2 (stalk). Binding to host cell’s cellular receptors happens through the S1 domain (see table 1 for a list of cell receptors used by human coronaviruses). The S2 domain promotes the fusion between the virion and host cell’s cell membranes. [3,5.]

The most abundant structural protein of a coronavirus particle is the transmembrane M protein (25–30 kDa). It interacts with other structural proteins to organize the assembly of the coronavirus particle. [3,3.]

The E protein (8–12 kDa) is an integral membrane protein found in small quantities in the viral envelope [3,3]. Inside the viral envelope, the N protein (43–50 kDa) binds the RNA genome to form a helical nucleocapsid [3,2–3].

Human coronavirus infection is initiated by virions binding to host cell receptors via their surface proteins. The viral entry occurs in a fusion between the virion and the cell membrane, followed by the uncoating, in which the viral genome is released into the cytoplasm - where the replication can now occur. The genomic RNA is synthesized into a full-length negative sense RNA, from which multiple nested messenger RNAs are later synthesized and translated into virus proteins. After the gene expression, the virions are assembled and transported to the plasma membrane and released outside the host cell by exocytosis. [3,5–7;6,437.]

2.3 Transmission and Clinical Features of HCoVs

The common human coronaviruses (HCoVs -229E, -NL63, -OC43 and -HKU1) circulate worldwide, and they are the second most common causative agents of upper respiratory tract infections [4]. HCoVs usually spread from person-to-person. An infected individual can transmit the virus to others, for example through close personal contact, through the air (by coughing or sneezing) and/or by transferring the virus to a surface. [7.]

Coronaviruses usually cause relatively mild upper respiratory tract infections in humans, with the exception of MERS, SARS and SARS-2 coronavirus infections, which can result in a more severe disease or death. Subclinical infections are also common. [4.] Typical symptoms of common human coronaviruses include: runny nose, sore throat, headache, cough and fever. They can also cause lower respiratory tract infections such as bronchitis and pneumonia. However, these are more prevalent among infants, the elderly and individuals with a weakened immune system. [7.] The immunity resulting from a coronavirus infection does not last long, and thus, reinfections are common [4].

3 COVID-19 Pandemic

3.1 Emergence of the COVID-19 Pandemic

In the 21st century infectious disease outbreaks – whether caused by re-emerging or novel pathogens, and whether transmitted by animals or by human-to-human – are increasingly occurring and spreading globally faster than ever [8,17]. This can be explained with factors such as biological, environmental and lifestyle changes [8,11]. Increased travel provides the opportunity for viruses to spread very quickly [9,278]. For instance, it took only around two months for the novel influenza virus, H1N1 pdm09, to reach all continents and to become a pandemic [8,15–17]. 70 % of emerging human pathogens come from animals [8,19], which can be explained by the increased close contacts between people and wildlife, livestock and domestic animals [8,25].

On December 31st, 2019, the Wuhan Municipal Health Commission published a media statement about a cluster of 'viral pneumonia' cases reported in Wuhan, China. Many of these cases shared a connection to Wuhan's Huanan Wholesale Seafood Market. On January 1st, 2020, the market was closed down since samples taken from the market tested positive for a novel coronavirus. The Chinese Center for Disease Control and Prevention stated on January 9th, that 15 of the 59 reported viral pneumonia cases also tested positive for a novel coronavirus. On the following day, the first genome sequence of the novel coronavirus was published. [10.] Although the first reported cases were from Wuhan, it cannot be ruled out that the virus could have circulated elsewhere prior to first findings [1,2].

The first confirmed death caused by the novel coronavirus was reported on January 11th, 2020 in China. The virus spread rapidly within China and by January 13th the first confirmed case outside of China was reported in Thailand. 11 days later the first European confirmed case was reported in France. On January 30th, only a month since the first report of the 'viral pneumonia' cases reported in Wuhan, the World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC) – WHO's highest level of alarm. [10.]

On February 11th, 2020, the novel coronavirus was officially named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV). On the same day WHO named the disease caused by

the virus as coronavirus disease 19 (COVID-19). A month later, on March 11th, WHO declared the outbreak a pandemic. Europe became the epicenter of the pandemic on mid-March 2020. [10.] Early timeline of the key events of the COVID-19 pandemic is shown in figure 2.

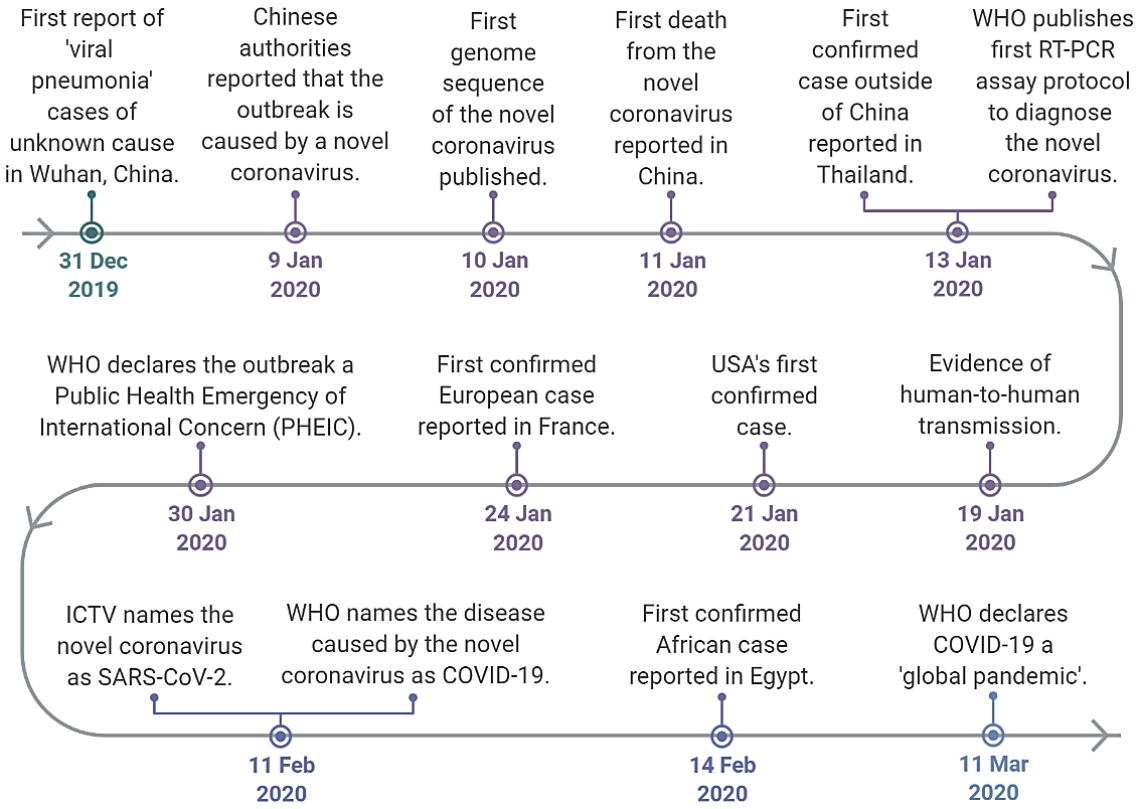

Figure 2. Key events of the early stage of the COVID-19 pandemic. Figure created with BioRender.com.

The pandemic situation worsened quickly as the virus continued to spread around the world. The number of recorded COVID-19 cases reached 100,000 on March 7th, 2020 [10] (week 10), and from there on both the reported cases and deaths started to grow significantly (see figures 3 and 4). Within a month the reported cases had increased tenfold, exceeding one million cases on April 3rd [10].

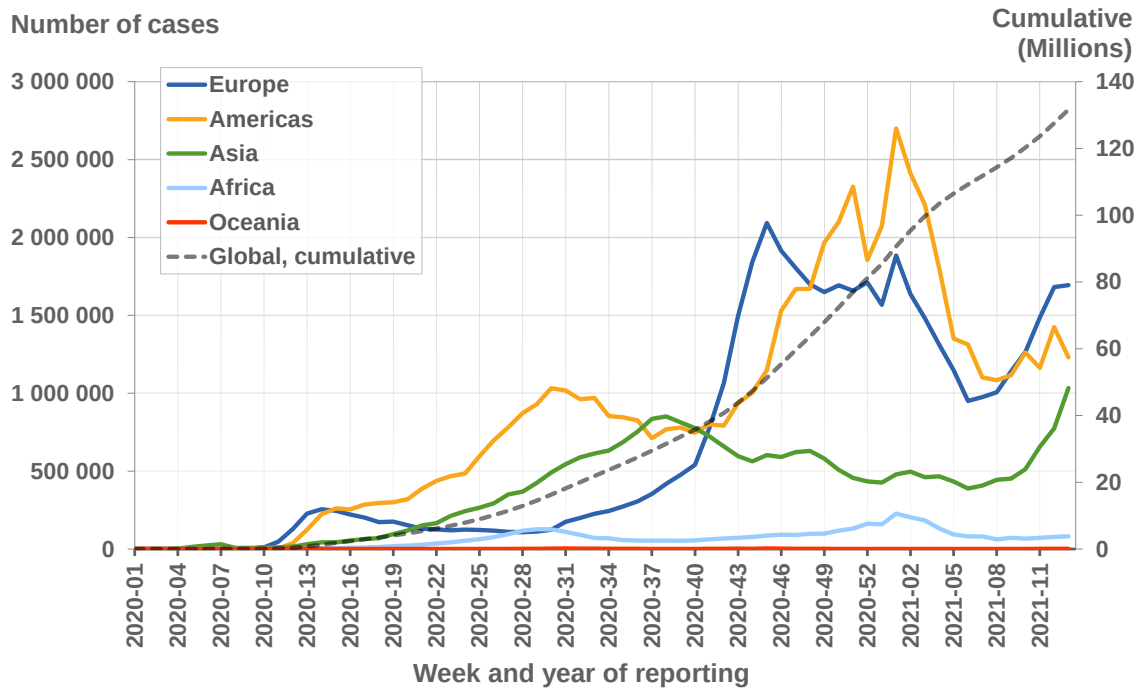

Figure 3. Distribution of reported COVID-19 cases worldwide, by continent, as well as the global cumulative total from January 2020 to March 2021. Data gathered from European Centre for Disease Prevention and Control (ECDC) (2021) [11].

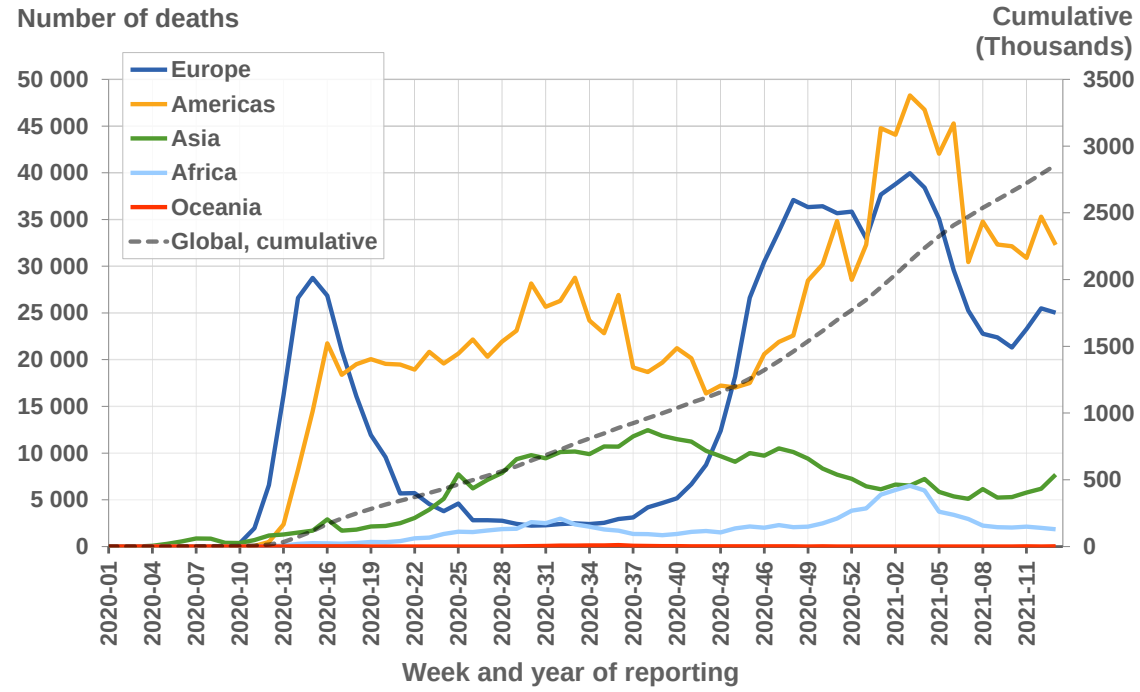

Figure 4. Distribution of reported COVID-19 deaths worldwide, by continent, as well as the global cumulative total from January 2020 to March 2021. Data gathered from ECDC (2021) [11].

It is important to note that the reported number of COVID-19 cases and deaths are somewhat approximate, since the local testing strategy and capacity and the efficacy of surveillance systems vary among countries, which affects the data. This partially explains the high variation in the number of cases and deaths among continents in proportion to their population. Especially in the early stages of the pandemic there was a lack of testing capacity which explains the relatively low number of recorded cases and deaths – more extensive testing will result in more cases being detected. [12.]

However, it is noticeable that the numbers of reported cases and deaths worldwide have been increasing steadily since the beginning of the pandemic (see global cumulative series on figures 3 and 4). It can also be noticed, that after the first wave, for example in Europe the pandemic situation improved significantly during the summer months of 2020. This was preceded by large scale governmental restrictions.

3.2 Controlling the COVID-19 Pandemic

There are many control measures that can help to manage outbreaks caused by a highly infectious virus. Because infections can be asymptomatic, social distancing and the use of protective equipment (for example masks) are effective precautions individuals can take to prevent the spread of the virus. More strict measures include isolating those infected and quarantining those exposed to the virus. In order for this to be effective, it is important to identify those who are infected. [9,286–287.]

An essential aspect of managing the COVID-19 pandemic has also been to identify as many individuals infected with SARS-CoV-2 as possible. Hence, there has been an urgent need for reliable *in vitro* diagnostics to detect SARS-CoV-2. The European Union Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices, currently applies to COVID-19 tests [13,1].

According to Directive 98/79/EC, IVDs must hold a CE marking when placed on the market within the European Union (EU). With affixing the CE marking, the manufacturer assures that the device meets the essential safety, health, design and manufacturing requirements laid out in the Directive 98/79/EC. [14.] The current IVD Directive is valid until May 25th, 2022, and as of May 26th, 2022 Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices comes into force [13,1;15,6].

Based on the grounds of protection of health, a national derogation from the conformity assessment procedures can be granted under the Directive 98/79/EC. The derogation means that a device may be placed on the market by the manufacturer, even if all the steps concerning the conformity assessment of the product have not been completed. The competent authority of an EU member country may grant this kind of derogation and it is effective solely in that member country. [13,1;15,8.] In Finland the competent authority is Fimea [16]. Often, the market access resulting from such derogation is provisional [15,8].

4 Objectives of the Project

The objective of this project was to collect data to be used in the evaluation of the clinical performance of Abacus Diagnostica's GenomEra® SARS-CoV-2 test (later frequently referred as index test). The purpose was to analyze samples in parallel with the GenomEra® SARS-CoV-2 test and THL's validated in-house real-time RT-PCR test, which served as the reference method. The clinical performance of the index test was evaluated through clinical sensitivity and specificity, inclusivity (= analytical reactivity) and cross-reactivity (= analytical specificity). The reproducibility of the index test was also considered to monitor day-to-day variability. Results of the GenomEra® SARS-CoV-2 test were compared with those obtained by the reference method.

5 Materials and Methods

The first section of this chapter gives a brief overview of the COVID-19 RT-PCR test process, from sample collection to test results, for both the index test and the reference test. The study population and specimen types used in this study are described in the second section, followed by sections three and four, which give more detailed descriptions of the method principles for both tests. The final section contains the biosafety regulations that were followed in this study.

5.1 COVID-19 RT-PCR Test Process

The steps of COVID-19 testing vary depending on the type of analysis to be conducted. For most nucleic acid amplification tests the process goes as described below.

A trained health care professional collects a swab sample from the respiratory tract of a suspect COVID-19 case and places the swab into a suitable viral transport medium. The clinical specimen is packaged and transported to a testing laboratory as ‘Biological Substance, Category B’ (UN3373) [17,1–4].

At a testing laboratory, the sample goes through an initial handling process inside a validated biological safety cabinet [17,1–2], which includes inactivation of a desired volume of the specimen. Next, if purified viral RNA is used as a starting material, RNA is extracted from the inactivated specimen. The purified viral RNA is reverse transcribed to complimentary DNA (cDNA) and amplified in polymerase chain reaction (PCR). If amplification is not observed, the sample does not contain the target virus and the test result is negative. If amplification is observed, the sample contains the target virus and the test result is positive (the target specific fluorescence signal rises above the predetermined threshold level). Usually with a real-time RT-PCR (also called quantitative RT-PCR, RT-qPCR), a cycle threshold (Ct) value is obtained for positive samples. Low Ct values indicate that high concentration of the target amplicon is present in the sample and high Ct values indicate lower concentrations of the target amplicon present in the sample.

Figure 5 demonstrates a simplified process diagram of the steps of COVID-19 testing – from sampling to final test results – for the index test and the reference test.

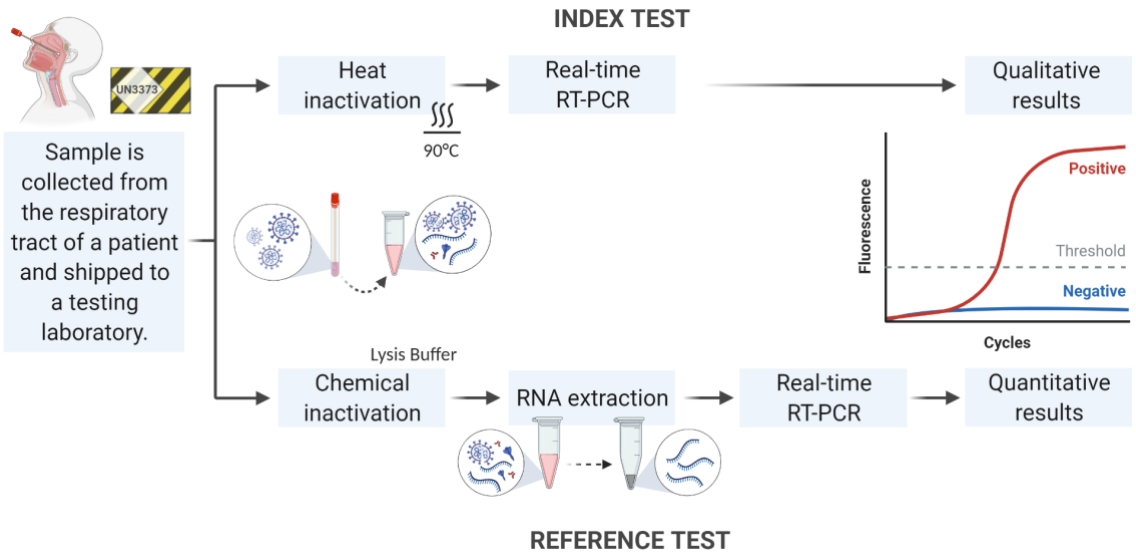

Figure 5. Workflow of the GenomEra® SARS-CoV-2 test (index test) and THL's in-house real-time RT-PCR test (reference test) for detecting SARS-CoV-2 viral RNA. Figure created with BioRender.com.

In the index and reference test methods, the test process proceeds in the same way up to the inactivation step. The index test utilizes heat for the inactivation while the reference method uses chemical inactivation. For the GenomEra® SARS-CoV-2 test, there is no need to perform a separate RNA extraction step prior to the PCR unlike there is for the reference test method. Test results obtained with the index test are qualitative and the results obtained with the reference method are quantitative.

5.2 Study Population and Specimen Types

The samples were selected based on which viral transport medium they had been collected in (suitable media included Copan eSwab, Copan Universal Transport Medium [UTM] or saline), and to cover temporally as many outbreak months as possible.

76 diagnostic upper respiratory swab samples from suspect COVID-19 cases were analyzed in this project. Of the 76 specimen, 37 had been collected into Copan UTM, 34 into clear liquid (which were assumed to be either saline or Copan eSwab), one into an unknown transport medium and four were dry stick samples. During the initial processing of the samples, the dry stick samples were placed into 1 ml phosphate buffered saline (PBS, pH 7.2, Gibco, Cat. No. 20012027). Two initially selected

samples (in clear liquid) were discarded from the study as they were collected from the same individuals already analyzed in this project.

For cross-reactivity studies, 30 (all in UTM) diagnostic upper respiratory samples containing known pathogens, 7 samples of isolated viral RNA and one sample of isolated bacterial DNA were studied. List of these pathogens is presented in subsection 6.4. For inclusivity studies two cultivated SARS-CoV-2 reference strains with geographical origins of Wuhan, China (Fin/1/20) and Milan, Italy (Fin/2/20) were tested. All the clinical COVID-19 samples had been collected in Finland in 2020. Summary of the selected samples is shown in table 2.

Table 2. Summary of specimens used in the evaluation of GenomEra® SARS-CoV-2 test's clinical performance. The table does not include the two discarded samples.

Specimen type and transport medium	SARS-CoV-2 positive	SARS-CoV-2 negative	Positive for other pathogens	Total
Nasopharyngeal swab, UTM	7	30	30	67
Nasopharyngeal swab, clear liquid	5	27	0	32
Nasopharyngeal swab, un-known media	0	1	0	1
Nasopharyngeal swab, dry stick	0	4	0	4
RNA/DNA	2	0	8	10
Total	14	62	38	114

At the beginning of the project, 55 samples were analyzed at two volumes (50 and 100 µl) with the GenomEra® SARS-CoV-2 test to determine if using a larger sample volume would increase the sensitivity of the test. No difference was observed in test results between the two volumes, and thus the remainder of the project was carried out using the sample volume of 50 µl.

For the reproducibility testing, Abacus Diagnostica provided identical sample panels for each participating study site. The samples included two SARS-CoV-2 positive samples with pre-determined concentrations of 2x and 3x limit of detection (LoD), and one SARS-CoV-2 negative sample.

5.3 Principle of GenomEra® SARS-CoV-2 Assay

The GenomEra® SARS-CoV-2 test is a rapid test for the qualitative detection of SARS-CoV-2 nucleic acid from respiratory swab samples. It amplifies and detects unique sequence regions of SARS-CoV-2 E and RNA dependent RNA polymerase (RdRP) genes (see table 3). Tests are performed on the GenomEra® CDX PCR system (Abacus Diagnostica, Order No. CDX-10-020) (figure 6) using the GenomEra® CDX software version 1.3.25 or above. The GenomEra® SARS-CoV-2 assay kit contains: test chips, buffer ampoules, sample processing control (SPC) tubes and a reusable chip holder. [18,3–4.]

Table 3. Oligonucleotides and their sequences used in the GenomEra® test for SARS-CoV-2 E and RdRP gene. Data gathered from Abacus Diagnostica [18,4].

Oligonucleotide	**Sequence (5'→3')**
E_F	CATCCGGAGTTGTTAATCCAGT
E_R	ACAAAGGCACGCTAGTAGTC
E_P	Red615-CGTCGGTTCATCATAAATTG-MGB-EDQ
RdRP_F	GTCACGGCCAATGTTAATGC
RdRP_R	TAAATTGCGGACATACTTATCGG
RdRP_P	FAM-CTACTGATGGTAACAAA-MGB-EDQ

The assay is intended to be used in a laboratory environment by laboratory personnel [18,3]. Four samples can be analyzed during a single run using microbe specific disposable test chips. All PCR reagents have been preloaded to the test chips, so there is no need for a cleanroom environment. The instrument heat seals the sample chips, which minimizes the risk of cross-contamination during an assay-run. In addition, it eases the waste disposal, as all the biohazardous waste is securely sealed. [19.] Assay run time for SARS-CoV-2 test chips is about 70 minutes [18,4].

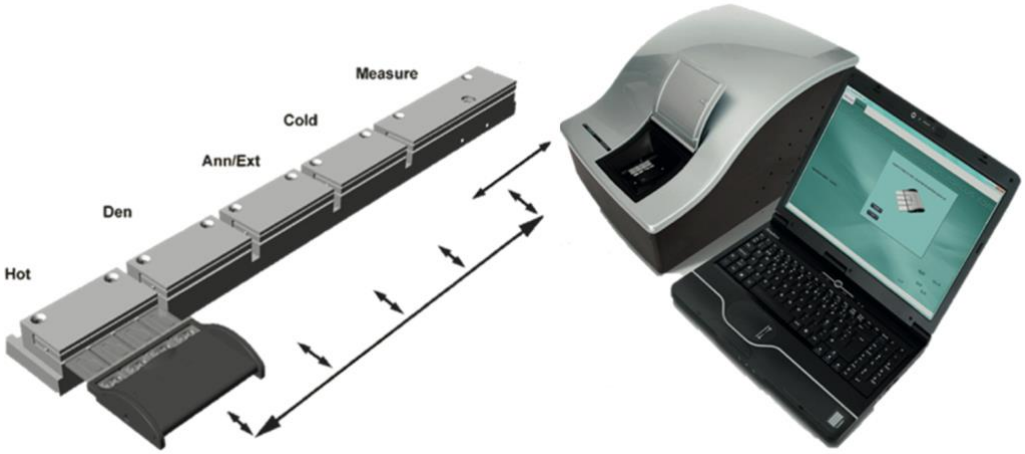

Figure 6. GenomEra® CDX PCR system consists an integrated thermal cycler and a time-resolved fluorometer [18,4]. Adapted from Abacus Diagnostica [19].

The test utilizes lanthanide chelate labelled oligonucleotide probes and time-resolved fluorescence technology (TRF) in the detection and measurement of the amplification products [20,5]. In TRF a pulsed light is used to excite the molecules in the sample, and a short delay is applied between the excitation pulse and the measurement of the emission signals (figure 7). Because of their long-lifetime emission properties, lanthanide chelates are used as fluorophores in TRF. The short delay after the excitation pulse allows the background fluorescence to decay to a negligible level before the measurement time window, increasing sensitivity along with the long decay times of lanthanide chelates. [20,5;21,477.]

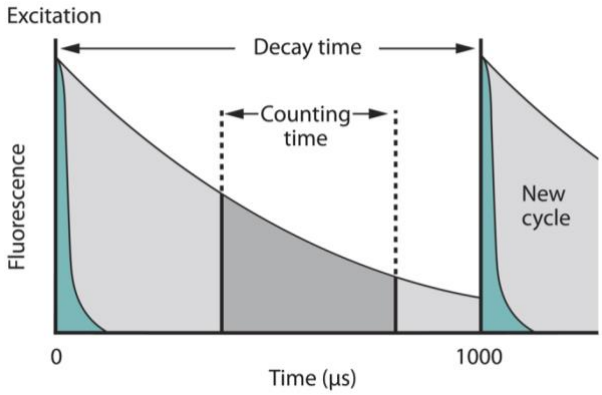

Figure 7. In time-resolved fluorescence, a short time delay is applied between the excitation pulses and the counting of emission signals. Copied from Abacus Diagnostica [19].

Sample preparation is completed inside a biological safety cabinet. 50 µl of the original specimen is pipetted into the SPC tube to rehydrate the sample processing control.

The specimen is inactivated by heating the SPC tube in a thermal block (Biosan Bio TDB-100) at 90 ºC for five minutes. During the heating step, RNA of the virus is released from its viral capsid. After the heating, a buffer ampoule is emptied into the SPC tube and mixed briefly by vortexing. Finally, 35 µl of the processed sample is pipetted into a test chip and the assay-run is started within three minutes. The sample preparation takes approximately 10–20 minutes. Preheated specimens can be stored in a refrigerator (+2 – +8 °C) for up to three hours prior analysis. [18,6.] The sample preparation process is illustrated in figure 8.

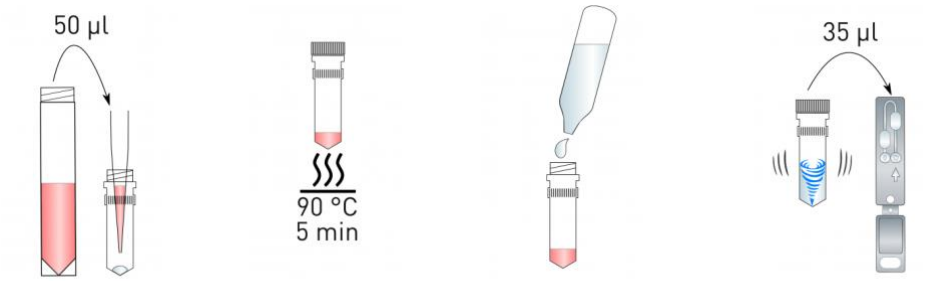

Figure 8. Sample preparation for the GenomEra® SARS-CoV-2 test. Copied from Abacus Diagnostica [22].

After an assay-run, qualitative results are obtained; a positive result indicates that the target sequence was found in the sample, a negative result indicates that the target sequence was not found in the sample and a borderline result indicates that the presence of the target sequence in the sample could not reliably be established making the result inconclusive. Results are given separately for both target genes (E and RdRP) and the sample is considered positive for SARS-CoV-2 if either one of the target genes is found. [18,7–8.] If neither of the target sequences, nor the sample processing control are detected, the results are announced as PCR inhibition [18,8].

5.4 Reference Method

THL's in-house real-time RT-PCR for SARS-CoV-2 E gene was used as the reference method. It uses primers and a FAM-labelled probe from a method published by Corman et al. and recommended by WHO [23]. E gene oligo mix (primers and probe by MetaBion, storage: ≤-18 °C) was prepared into nuclease-free water (Sigma, Cat. No. W4502, storage: ≤-18 °C) as shown in table 4. A dilution of purified SARS-CoV RNA

(10^-5, storage: ≤-18 °C [stock stored at -70 °C]) served as the positive PCR control and nuclease-free water as the negative PCR control.

Table 4. Oligonucleotides used in the SARS-CoV-2 E gene real-time RT-PCR. Data gathered from Corman et al. (2020) [24,2].

Component	**Concentration (µM)**	**Volume (µl)**	
Oligonucleotide (100 µM)			**Sequence (5'→3')**
E_Sarbeco_F1	1.4	14	ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2	1.4	14	ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1	0.7	7	FAM-ACACTAGCCATCCTTCCTGCGCTTCG-BHQ1
Nuclease-free water		965	
Total		1,000	

Quantabio 2x qScript® XLT One-Step RT-qPCR ToughMix® (VWR, Cat. No. 733-2230, storage: ≤-18 °C) was used as the reaction master mix [25]. It has the RT and the PCR enzymes in the same mix, which allows for the reverse transcription and PCR amplification to occur in one reaction tube. The reaction setup is presented in table 5.

Table 5. SARS-CoV-2 E gene real-time RT-PCR reaction setup. Volumes for one reaction.

Component	**Volume (µl)**
Quantabio 2x qScript® XLT One-Step RT-qPCR ToughMix®	12.5
E gene oligo mix	7.5
Template RNA	5
Total	25

The real-time RT-PCR was performed in the BioRad® CFX96™ qPCR instrument. The thermal cycling protocol is described in table 6.

Table 6. Thermal cycling protocol used in the SARS-CoV-2 E gene real-time RT-PCR.

Step	**Temperature (°C)**	**Time**	**No. of Cycles**
Reverse transcription	55	20 min	1
Polymerase activation	95	3 min	1
Denaturation	95	15 sec	45
Annealing/Extension	58	1 min	45

Prior to the PCR, the viral RNA was extracted from the specimen with PerkinElmer chemagic360 instrument, using the Chemagic™ Viral300 DNA/RNA Kit (PerkinElmer, Cat. No. CMG-1033-ST) and chemagic Viral300 360 H96 drying prefilling VD141211.che protocol. The instrument utilizes metal rods for magnetic separation of nucleic acids. All the necessary reagents needed for the extraction process are provided in the Chemagic™ Viral300 DNA/RNA Kit. In addition, ultrapure water (LATU, THL), 70 % ethanol (Spiritus Fortis 70 %, Berner Oy, Cat. No. 32. 13210424) and 10 % chloramine-T (Klorilli, KiiltoClean Oy, Cat. No. 50484) are needed for cleaning the instrument.

Before the extraction, 100 µl of the specimen was lysed with 350 µl of Buffer RLT (RNEasy Mini kit, Qiagen, Cat. No. 74106) for 10 minutes. To inactivate RNases in the lysate, 1 % v/v 2-Mercaptoethanol (Sigma-Aldrich, Cat. No. M3148) was added to the Buffer RLT prior to the addition of the specimen [26].

5.5 Biosafety and Chemical Safety

The European Commission has classified SARS-CoV-2 as a risk group 3 biological agent [27]. All laboratory work was carried out by following the national guidelines on laboratory biosafety. Additional heightened safety measures listed in THL's risk assessment concerning biosafety level 2 (BSL-2) laboratory work related to SARS-CoV-2 were also followed.

Personal protective equipment included a long-sleeved laboratory coat, disposable sleeve covers and two layers of protective disposable gloves (nitrile and latex). Initial processing of the samples (before inactivation) was conducted in a validated biological safety cabinet. All handling of samples was carried out carefully and the possibility of a simultaneous occurrence of other pathogens was taken into account.

10 % chloramine-T ($C_7H_7ClNO_2SNa$) dilution and 70 % ethanol (C_2H_5OH) were used as disinfectants, with contact times of 10–15 minutes and one minute, respectively. Chloramine-T and ethanol act by denaturing proteins from enveloped viruses [17,3]. All infectious waste generated during the study was inactivated by autoclaving at 121 °C for 40 minutes [28].

Substances used in this study that are classified as hazardous under the European Union Chemicals Legislation are listed in appendix 1. The hazardous chemical waste was collected into labelled containers and transported to the specialized disposal plant according to THL's laboratory guidelines.

6 Results

The results presented in this report are only a segment of the results that were used for the actual evaluation of the clinical performance of the GenomEra® SARS-CoV-2 assay. Therefore, the statistical measures presented are only estimates of the clinical performance of the test.

6.1 Clinical Sensitivity and Specificity

Total of 12 SARS-CoV-2 positive and 62 SARS-CoV-2 negative samples were tested to estimate clinical sensitivity and specificity of the GenomEra® SARS-CoV-2 assay (table 7).

Table 7. PCR results of the index and reference methods for samples used to assess the clinical sensitivity and specificity of the GenomEra® SARS-CoV-2 test.

GenomEra® SARS-CoV-2 (E and RdRP genes)	Reference method (E gene)		
	Positive	**Negative**	**Total**
Positive	10	0	10
Negative	2	59	61
Inhibition	0	3	3
Total	12	62	74

Clinical sensitivity describes the ability of a test to correctly identify positive samples. It is calculated by dividing the number of positive samples correctly identified by the index test with the number of all positive samples. Estimation of the clinical sensitivity of the index test was calculated from 12 SARS-CoV-2 positive samples. Table 8 shows the results obtained with the index test and the reference method, as well as the Ct values from the reference method.

Table 8. PCR results for the positive samples used to assess the GenomEra® SARS-CoV-2 test's clinical sensitivity.

Sample #	GenomEra® SARS-CoV-2 (E and RdRP genes)	Reference method (E gene)	Reference method Ct value (E gene)
30-001	Positive	Positive	19.66
30-002	Positive	Positive	24.35
30-005	Negative	Positive	34.64
30-030	Negative	Positive	38.95
30-118	Positive	Positive	30.99
30-119	Positive	Positive	34.15
30-120	Positive	Positive	30.04
30-121	Positive	Positive	33.36
30-122	Positive	Positive	31.06
30-123	Positive	Positive	30.38
30-127	Positive	Positive	29.25
30-129	Positive	Positive	30.27

GenomEra® SARS-CoV-2 test identified correctly 10 out of the 12 positive samples tested. False negative results were obtained from samples with the highest Ct values; 30-005 with a Ct value of 34.64 and 30-030 with a Ct value of 38.95. From these results, the estimation of the index test's clinical sensitivity was calculated to be 83.3 %.

Sensitivity = True Positives / (True Positives + False Negatives) x 100 % = 10 / (10 + 2) = **83.3 %**.

Clinical specificity describes the ability of a test to correctly identify negative samples. It is calculated by dividing the number of correctly identified negative samples by the total amount of negative samples. 62 SARS-CoV-2 negative samples were used to estimate the clinical specificity of GenomEra® SARS-CoV-2 test. The test identified correctly 59 of the 62 negative samples tested. For three of the samples, the test gave inhibitory results.

Of the three samples resulting in PCR inhibition, one had been collected into UTM, and two into clear liquid. The sample collected into UTM could not be retested because there was not enough of the original specimen left, and therefore it was excluded from the calculations. The two samples that had been collected into clear liquid were retested to see, if the inhibitions were due to mistakes in pipetting the processed sample into the test chip, or if the transport medium could have been unsuitable. The

re-tests also resulted in PCR inhibition, so presumably the inhibitions were due to an unsuitable transport media. These two were also excluded from the calculations. From these results, the estimation of clinical specificity of the index test was calculated to be 100 %.

Specificity = True Negatives / (True Negatives + False Positives) x 100 % = 59 / (59 + 0) = **100 %**.

6.2 Reproducibility

Reproducibility of the GenomEra® SARS-CoV-2 test was studied by assessing between-days variability of the test; three samples were analyzed on five separate days. Reproducibility samples consisted of the following: 2x LoD and 3x LoD SARS-CoV-2 samples and a negative sample. Samples were stored at +4 °C between runs. The results are shown in table 9.

Table 9. Results of reproducibility testing of the GenomEra® SARS-CoV-2 test. A plus sign (+) refers to positive result and correspondingly a minus sign (-) refers to negative result.

Sample	Day 1	Day 2	Day 3	Day 4	Day 5	Total
2x LoD	+	+	+	+	+	5/5
3x LoD	+	+	+	+	+	5/5
Negative	-	-	-	-	-	5/5

GenomEra® SARS-CoV-2 test correctly identified the 2x LoD and 3x LoD SARS-CoV-2 samples as positive on all five days. The negative sample was also correctly identified as negative on all five days. This indicates that the reproducibility of the GenomEra® SARS-CoV-2 test is excellent.

6.3 Inclusivity

For the inclusivity studies two cultivated SARS-CoV-2 reference strains with known geographical origin were tested to see if the GenomEra® SARS-CoV-2 test detects both strains equally well. The strains were Fin/1/20 and Fin/2/20 with the geographical origins of Wuhan, China and Milan, Italy, respectively.

The concentrations of the original Fin/1/20 and Fin/2/20 RNA stocks were 3.8x10^7 and 4.0x10^8 viral genomic copies per 5 µl. Ten-fold serial dilutions were prepared from the stock-RNAs of both SARS-CoV-2 strains into nuclease-free water. In both cases, the initial 1:10 dilution was prepared by adding 10 µl of stock-RNA to 90 µl of nuclease-free water.

Testing was started by analyzing four replicate samples at a dilution of 1:10^4. If all four did not give a positive result, a concentration of 10 times higher was tested. If all four were positive, a ten-fold lower concentration was tested. At the end, the dilution resulting in the lowest four out of four positive samples was reported as the detection limit.

For both strains, a 1:10^4 dilution yielded four out of four positives. With a 1:10^5 dilution, the Wuhan strain gave one positive out of four, and the Milan strain gave four out of four positives. Thus, the detection limit of the Wuhan strain was determined to be 3,800 gene copies per 5 µl of the RNA dilution. The Milan strain gave zero out of four positives at a 1:10^6 dilution. Thus, the detection limit of the Milan strain was determined to be 4,000 gene copies per 5 µl of the RNA dilution. The results are presented in table 10.

Table 10. Results of the inclusivity studies of two geographically differing SARS-CoV-2 strains. Copy quantities per reaction are calculated using the reference method. A plus sign (+) refers to positive result and correspondingly a minus sign (-) refers to negative result.

Strain	RNA dilution	Copies per 5 µl of RNA dilution	Reference method Ct value (E gene)	GenomEra® SARS-CoV-2 PCR result			
				E gene	RdRP gene	E and RdRP genes	No. of positives
Fin1/20, Wuhan, China	$1:10^4$	3,800	30.99	+	+	Positive	4/4
				+	+	Positive	
				+	+	Positive	
				+	+	Positive	
	$1:10^5$	380	–	-	-	Negative	1/4
				+	-	Positive	
				-	-	Negative	
				-	-	Negative	
Fin2/20, Milan, Italy	$1:10^4$	40,000	27.22	+	+	Positive	4/4
				+	+	Positive	
				+	+	Positive	
				+	+	Positive	
	$1:10^5$	4,000	30.69	+	+	Positive	4/4
				+	+	Positive	
				-	+	Positive	
				-	+	Positive	
	$1:10^6$	400	–	-	-	Negative	0/4
				-	-	Negative	
				-	-	Negative	
				-	-	Negative	

No difference was observed in the ability of the GenomEra® SARS-CoV-2 test to detect the two geographically differing SARS-CoV-2 strains. In both cases the lowest four out of four positives was obtained at a Ct value slightly below 31: 30.99 for the Wuhan strain and 30.69 for the Milan strain. The Ct values and copy quantities per reaction were obtained by the reference method.

A series of predetermined SARS-CoV-2 positive samples covering the early part of the COVID-19 pandemic, from February to June 2020, were also tested to determine whether the temporal variation in the circulation of the virus has an effect on the ability of the GenomEra® SARS-CoV-2 test to detect the virus. All samples were tested in duplicate. The results are presented in table 11.

Table 11. Results of the inclusivity studies using temporally varying SARS-CoV-2 strains. A plus sign (+) refers to positive result and correspondingly a minus sign (-) refers to negative result.

Sample #	Sample collected (week)	Reference method PCR result	Reference method Ct value	GenomEra® SARS-CoV-2 PCR result		
		E gene		E gene	RdRP gene	E and RdRP genes
30-118	11	Positive	30.99	+	+	Positive
				+	+	Positive
30-127	12	Positive	29.25	+	+	Positive
				+	+	Positive
30-119	13	Positive	34.15	+	-	Positive
				-	+	Positive
30-120	14	Positive	30.04	+	+	Positive
				+	+	Positive
30-122	15	Positive	31.06	+	-	Positive
				-	+	Positive
30-121	16	Positive	33.36	-	-	Negative
				+	-	Positive
30-123	17	Positive	30.38	-	+	Positive
				+	+	Positive
30-129	18	Positive	30.27	+	+	Positive
				+	+	Positive
30-005	19	Positive	34.64	-	-	Negative
				-	-	Negative
30-002	20	Positive	24.35	+	+	Positive
				+	+	Positive
30-001	20	Positive	19.66	+	+	Positive
				+	+	Positive
30-030	22	Positive	38.95	-	-	Negative
				-	-	Negative

The index test gave negative results on samples 30-005 and 30-030, from weeks 19 and 22, with Ct values of 34.64 and 38.95, respectively. Most likely this does not result from the temporal variation of the virus, but instead from the low viral load present in these samples – as can be seen from the high Ct values. Overall, no difference was observed in the ability of the GenomEra® SARS-CoV-2 test to detect the virus in terms of temporal variation.

6.4 Cross-reactivity

The cross-reactivity of the GenomEra® SARS-CoV-2 test was evaluated by testing a panel of potentially cross-reacting micro-organisms (potential to cause false positive results); 16 pathogenic micro-organisms known to cause respiratory tract infections in humans were selected based on the guidelines provided by WHO in the Emergency Use Listing of IVDs [29].

The sample panel consisted of 30 previously collected clinical respiratory swab samples, seven viral RNAs and one bacterial DNA. Samples were stored at -70 °C and were thawed prior analysis. Preparation of the clinical samples was conducted in the same manner as is demonstrated in subsection 5.3. The viral RNA and bacterial DNA samples were prepared by spiking 50 µL of PBS with 5 µL of the RNA/DNA. All cross-reactivity samples were tested in duplicate. Analysis results are presented in table 12.

Table 12. Results of the cross-reactivity studies of the GenomEra® SARS-CoV-2 test.

Organism	**No. of clinical samples**	**No. of isolated RNA**	**Detected amplifications**	
			Reference method (E gene)	**GenomEra® SARS-CoV-2 (E and RdRP genes)**
human coronavirus 229E	4	-	0/4	0/4
human coronavirus NL63	4	-	0/4	0/4
human coronavirus HKU1	4	-	0/4	0/4
human coronavirus OC43	4	-	0/4	0/4
Middle East respiratory syndrome-related coronavirus (MERS-CoV)	-	1	0/1	0/1
severe acute respiratory syndrome coronavirus (SARS-CoV)	-	1	1/1	0/1
human adenovirus	2	-	0/2	0/2
human metapneumovirus	-	2	0/2	0/2
human parainfluenza virus 1	-	1	0/1	0/1
influenza A virus	4	-	0/4	0/4
influenza B virus	4	-	0/4	0/4
human respiratory syncytial virus	2	-	0/2	0/2
rhinovirus	2	-	0/2	0/2
human parechovirus 3	-	1	0/1	0/1
enterovirus D68	-	1	0/1	0/1
Mycoplasma pneumoniae	-	1 DNA	0/1	0/1

No cross-reactivity was observed with the GenomEra® SARS-CoV-2 test. THL's in-house real-time RT-PCR test gave false positive result for SARS-CoV. However, this was expected, since the in-house E gene PCR assay is designed to detect both SARS-CoV and SARS-CoV-2, respectively.

7 Conclusions

Overall, the study proceeded well, although a few minor challenges did occur – the most significant being the paucity of positive SARS-CoV-2 samples. This was due to Finland's excellent epidemic situation in the summer of 2020, when there was very little SARS-CoV-2 circulating. Only 12 SARS-CoV-2 positive samples were tested, from which two (those with the lowest viral loads) gave false negative results. Based on those twelve samples the estimation of the clinical sensitivity was determined at 83.3 %. The clinical specificity of the test proved excellent; all SARS-CoV-2 negative samples were correctly identified (100 %). The index test identified all tested SARS-CoV-2 positive samples equally well, regardless of the temporal variation of the virus or the geographical origin of the strain. The index test did not give false positive results for any of the potentially cross-reacting micro-organisms tested. Since no cross-reactivity was observed, it indicates that Abacus Diagnostica's oligonucleotides used in the GenomEra® SARS-CoV-2 test are highly specific for detecting only SARS-CoV-2 RNA.

The statistical measures presented in this paper are only estimates of the index test's clinical performance and should not be considered indicative of the actual performance values (a larger number of samples should be tested in order to produce reliable results). The final clinical performance of Abacus Diagnostica's GenomEra® SARS-CoV-2 test was evaluated on the basis of the results obtained at three different study sites. The GenomEra® SARS-CoV-2 assay was CE-IVD approved for the European market on 17th of July, 2020 [30].

The index test does not necessitate a specific cleanroom laboratory environment or various sets of reagents and laboratory instruments, which makes executing the assay extremely effortless and straight-forward. In addition, since no RNA extraction is required, the results are obtained very quickly - the turnaround time is only approximately 1.5 hours.

In this evaluation, very weak SARS-CoV-2 positive samples, detected by the reference method, remained negative. In acute COVID-19 infection the virus amount is usually extremely high and thus the sensitivity of the index assay will not be limiting. A more sensitive PCR assay may be needed to detect the virus for example in asymptomatic cases. With the index test, four samples can be analyzed during one assay-run, hence, it is not ideal for situations where large amounts of samples need to be analyzed in a short time period. It should also be noted that some viral transport media are incompatible with the index test, and may inhibit PCR testing.

In conclusion, the results indicate that Abacus Diagnostica's GenomEra® SARS-CoV-2 assay is a reliable test for the qualitative detection of SARS-CoV-2 RNA from nasopharyngeal swab samples collected in UTM, Copan eSwab or saline. Due to the ease of use, it makes the assay especially suitable for environments where results are needed quickly and effortlessly, such as hospitals or environments where extensive laboratory equipment is not accessible.

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Hazardous Substances Used in This Project

Substances used in this project that are classified as hazardous under the European Union Chemicals Legislation:

- Klorilli (KiiltoClean Oy, Cat. No. 50484) 
  - chloramine T (CAS: 127-65-1)
  - sodium metasilicate pentahydrate (CAS: 10213-79-3)
  - sodium alkyl sec. sulfonate (CAS: 97489-15-1) [31]
- Spiritus Fortis 70 % (Berner Oy, Cat. No. 32. 13210424) 
  - ethanol (CAS: 64-17-5) [32]
- 2-Mercaptoethanol (Sigma-Aldrich, Cat. No. M3148) 
  - β-Mercaptoethanol (CAS: 60-24-2) [33]
- Buffer RLT (Qiagen, RNEasy Mini Kit, Cat. No. 74106) 
  - guanidium thiocyanate (CAS: 593-84-0) [34].