COVID-19 Tests Project @ BI
Comparison of swab vs Salivette® Cortisol

Programme to identify SARS-CoV-2/COVID-19 infection with RT-qPCR using a nasopharyngeal swab vs saliva collected with Salivette® Cortisol

Boehringer Ingelheim

Test protocol

Document date:	28 July 2020
BI Project No	**0352-2150**
Title	**COVID-19 tests: a systematic comparison of viral RNA test sample taking for RT-qPCR, in this case with a nasopharyngeal swab or saliva collected with Salivette® Cortisol, to diagnose SARS-CoV-2 virus infection**
Short title	**COVID-19 tests: nasopharyngeal swab vs saliva collected with Salivette® Cortisol for viral RNA sampling for RT-qPCR**
Area of investigation	COVID-19
***In vitro* diagnosis compared in the test programme**	• **Kylt® for detection/confirmation of SARS-CoV-2 (PCR)** [redacted] • **cobas® SARS-CoV-2 (PCR) test** [redacted] • **TaqPath™ COVID-19 CE-IVD RT-PCR kit** [redacted] • Salivette® Cortisol
Sampling methods	1. **Swab – by the medical staff** 2. **Salivette® Cortisol – by the participant**
Global Programme [redacted]	[redacted] Telephone: [redacted] Email: [redacted]
Local Programme [redacted]	[redacted] **Telephone:** [redacted] **ext** [redacted] **/** [redacted] **Email:** [redacted]

Coordinating Investigator	[redacted] ext [redacted] 24-hour telephone [redacted] Email: [redacted]	
[redacted] **Laboratory Analyst (internal)**	[redacted] Telephone: [redacted] Email: [redacted]	
Laboratory (external)	[redacted] *Telephone:* [redacted] *Email:* [redacted]	
[redacted] **Statistician**	[redacted] Telephone: [redacted] Email: [redacted]	
Status	Protocol description document	
Version and date	Version: 1.0	Date: 28 July 2020

PROTOCOL SUMMARY

Company name	Boehringer Ingelheim (BI)
Protocol date	*28 July 2020*
Revision date	Not applicable
BI project number	0352-2150
Programme title	**COVID-19 tests @ BI: a systematic comparison of viral RNA test sample taking for RT-qPCR, in this case with a nasopharyngeal swab or saliva collected with Salivette® Cortisol, to diagnose SARS-CoV-2 virus infection**
Coordinating Investigator:	[redacted] ext [redacted] 24-hour telephone [redacted] Email: [redacted]
Test sites	[redacted]
Laboratory sites	[redacted]
Area of investigation	COVID-19
Justification for the programme	In order to have a correct diagnosis for individuals infected with SARS-CoV-2, reliable diagnostic tools must be identified, selected and regularly used. All results must be interpreted in relation to medical history and clinical evidence. RT-qPCR is the standard for identifying SARS-CoV-2 infection using viral RNA detection, generally with medical staff taking nasopharyngeal samples. This is considered to be an established procedure and is expected to provide reliable results; however, it has been found that the procedure depends on participant cooperation and staff experience. Compared to the previous technique, self-sampling is not considered optimal and may produce false-negative results due to the complex collection techniques and confounding human factors. In some cases it is difficult or impossible to obtain suitable samples. Self-sampling can lead to a lower risk of spreading the virus, as no visits to special centres are required. Furthermore, the biosafety level and risk of infections of medical staff when taking swab samples are reduced significantly.
Objective of the programme	The objective of the comparison is to demonstrate that saliva collection with Salivette® Cortisol is as reliable as the use of throat swabs in sampling viral RNA for RT-qPCR and identifying SARS-COV-2 infections. PCR for RNA detection of SARS-CoV-2 in throat swab samples and saliva samples collected with Salivette® Cortisol BI internal laboratories Certified external laboratories

Programme design	Parallel tests of different sample matrices
Number of participants	Total number: minimum of 20 and up to 80 participants who are positive for SARS-CoV-2
Main inclusion criteria for the pilot phase	COVID-19 diagnosis confirmed by PCR (within the past 5 days)
***In vitro* diagnosis**	• **Kylt® for detection/confirmation of SARS-CoV-2 (PCR)** [redacted] • **cobas® SARS-CoV-2 (PCR) test** [redacted] • **TaqPath™ COVID-19 CE-IVD RT-PCR kit** [redacted]
Day of the test	One single sample is planned
Statistical methods	Descriptive statistics directly comparing the test results

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ABBREVIATIONS

BI	Boehringer Ingelheim
CA	Competent authority
COVID-19	Coronavirus disease 2019
GCP	Good Clinical Practice
ICF	Informed consent form
Ig	Immunoglobulin
IPC	Internal positive control
IU	International units
K	Dispersion factor
LLOQ	Lower limit of quantification
MERS	Middle East Respiratory Syndrome
OD	On-demand
PI	Package insert
PoC	Point of care
RNA	Ribonucleic acid
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
ULN	Upper limit of normal
ULOQ	Upper limit of quantification
UTM	Universal Transport Medium
WFH	Work from home
WHO	World Health Organization

1. INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been identified as the cause of the pandemic outbreak of the coronavirus disease 2019 (COVID-19). Boehringer Ingelheim (BI) has launched a large-scale RT-PCR confirmation programme called COVID-19 Tests to counter the pandemic and safeguard public health. This dedicated health care measure was developed to complement the actions implemented by public health care to relieve the efforts of the health authorities.

The global pandemic COVID-19 is a reportable disease in most countries and local health authorities are monitoring the contacts of infected individuals to detect potential additional infections in order to prevent the spread. Contact tests generally depend on the presence of certain respiratory symptoms which coincide with the established risk criteria for increasing the probability of a positive nucleosides (RNA) result for SARS in swabs or sputum samples **[RKI 2020]**. It is therefore assumed that tests must be performed on as many individuals as possible in order to contain the pandemic situation. As such, the sampling method has become a critical factor, as it is more difficult to take swab samples than to collect saliva, and sampling must be optimised to allow more flexibility in obtaining samples for virus RNA identification, regardless of the availability of medical staff and testing centres. Furthermore, a method that could be performed at home and during quarantine will prevent the spread and reduce the biosafety risk in general.

1.1 MEDICAL BACKGROUND

SARS-CoV-2 belongs to the *Coronaviridae* family, which also includes members that cause the common cold, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) **[Liu et al. 2020]**.

It has been confirmed that the novel coronavirus is transmitted from person to person. Close contact between individuals (< 1.5 m) promotes the spread of the virus and, as a result, the disease. It is spread predominantly through droplets expelled when coughing or sneezing, but also through atomised particles when speaking. It must be assumed that transmission takes place when kissing, shaking hands or touching contaminated surfaces in public spaces (public transport, door handles, buttons, etc.), particularly when touching the mouth, nose or eyes afterwards. Given the many uncertainties regarding SARS-CoV-2 infection to date, including the dispersion factor (K), it is important to closely monitor the social setting and conduct extensive tests to control the pandemic situation.

COVID-19 symptoms can vary from very mild to severe and may include fever, cough, breathing difficulty and fatigue, which commonly occur after an incubation period of 2 to 14 days **[Huang et al. 2020]**. These symptoms can become serious and include fatal outcomes. It is thought that approximately 20% of infected patients show non-specific symptoms or no symptoms at all, but can still spread the virus **[He et al. 2020].** Unless they are quickly identified by means of sufficient, potentially regular off-site detection activities, these individuals have a high risk of unwittingly transmitting SARS-CoV-2, which is a significant threat to public health against a backdrop of acute infection driven by an on-demand test scheme. It is understood that the burden of regular tests cannot be applied to all, but rather to selected groups.

There is not yet a vaccine available for SARS-CoV-2, nor are there any specific antiviral drugs for COVID-19. The standard treatment is targeted primarily at relieving the symptoms, while compassionate use of medications is limited mainly to clinical studies, as well as invasive measures to save lives in severe cases, which often require long-term intensive care. The long-term impact of

COVID-19 on health cannot be evaluated at this early stage of the disease, but emerging evidence suggests that the infections can cause serious harm and potentially cause sequelae with a negative impact on overall health **[RKI 2020]**. As such, a test strategy with the diagnostic tools available for prevention or early diagnosis of COVID-19 is essential as an additional measure to the rules in place, such as social distancing and hygiene to prevent uncontrolled spread, to prevent the health services from collapsing and to reduce the long-term impact on public health.

1.2 DIAGNOSTIC PROFILE

The standard laboratory test for diagnosing SARS-CoV-2 is an RT-qPCR certified protocol to identify the RNA of the virus in swab samples (nasopharyngeal/oropharyngeal) or sputum samples **[Udugama et al. 2020]**.

1.2.1 Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RT-qPCR is a bioanalytical method for detecting nucleosides (DNA or RNA) in a wide range of test samples (e.g. blood, saliva and swabs). The technique is based on the *in vitro* amplification of genetic material using complementary nucleoside fragments (primers) and specific enzymes (polymerases). While the original material is undetectable due to its low concentration, the amplification can easily be detected, e.g. by means of gel electrophoresis or, in the case of RT-qPCR, automatically by digitally measuring a fluorescent signal in the device. There is currently a wide range of modified RT-qPCR techniques available for various situations. For RNA detection (SARS-CoV-2 genome), another specific enzyme is required: reverse transcriptase (RT). There is a RT-PCR protocol available from Charité Virology for diagnosing COVID-19 **[Corman et al. 2020]**. The **Kylt® for detection/confirmation of SARS-CoV-2** (PCR) kit is based on the PCR method published by Corman et al., which detects specific E gene sequences, and specific sequences in the second PCR of the RdRP gene and the S gene of SARS-CoV2.

A second test kit with RT-PCR is the TaqPath™ COVID-19 CE-IVD RT-PCR kit, provided by [redacted], which uses three different specific sequences of SARS-CoV-2 in the ORF1ab, N and S genes of the virus, for a multiplex detection focus.

The cobas® SARS-CoV-2 (PCR) test, by [redacted], is a PCR detection system which is fully automated for the cobas® 6800/8800 laboratory systems. This PCR detects in parallel a fragment of the E gene of the pan-Sarbecovirus and a specific ORF1 sequence of SARS-CoV-2, and is validated for nasopharyngeal and oropharyngeal samples taken with swabs and collected in UTM.

The three PCR kits include an internal positive control (IPC) which targets the RNA of human β-actin as an endogenous or externally added control, or externally added control targets in each reaction to validate the polymerase reaction.

1.3 JUSTIFICATION

In order to effectively test and monitor, reliable diagnostic tools must be selected, implemented and regularly performed. The results must be evaluated in relation to the medical history and clinical evidence. RT-qPCR is the standard for identifying SARS-CoV-2 infection and for detecting viral RNA, generally when the medical staff take nasopharyngeal swab samples. This is considered to be an established procedure and is expected to provide reliable results; however, it has been found that the procedure depends on participant cooperation and staff experience. Furthermore, self-sampling is not considered optimal and may produce false-negative results due to the difficulty of the technique

and the high level of variability due to human factors. In some cases it is difficult or impossible to obtain suitable samples. Furthermore, as there is no need to go to special centres, there is a lower risk of spreading the virus. It also significantly reduces biosafety and risk of infections for medical staff when taking swab samples.

The Salivette® Cortisol device will be used to collect saliva from the patient due to its high capacity to stimulate salivation and absorb saliva. In this saliva sample, it is expected to find SARS-CoV-2 viral RNA which will be identified through processing with the RT-qPCR technique, as described for the nasopharyngeal mucosa swab samples. No cortisol measurements will be performed in the collected sample.

1.4 RISK-BENEFIT ASSESSMENT

The Salivette® Cortisol device, which is placed in the mouth and gently chewed for approximately 60 seconds, is considered to be very low risk. Its taste may be considered unpleasant. It is handled by the participant and, as such, there is no additional risk expected for the medical staff. During the tests, everyone wears masks in the rooms while the swab samples are taken, and the medical staff are protected with FFP2 masks, protective clothing and double gloves. Sampling with Salivette® Cortisol can be performed on-site. The layout of the room will follow the general distancing and hygiene measures in place, and surfaces will be cleaned and disinfected in line with procedure after each participant leaves. The risk of harm, such as sequelae due to the medical procedure, and the risk of infection of all participants, including the occupational health care team, was assessed before the tests and was considered highly unlikely.

There is recent evidence (Wyllie, AL et al. 2020. https://doi.org/10.1101/2020.04.16.20067835doi) that saliva sampling potentially produces more reliable results when compared to swab sampling.

Conclusions: It is considered necessary to establish sampling methods other than swab samples ion order to reduce the biosafety risk and increase the flexibility of tests and their acceptance among potentially infected individuals, as taking swab samples is more invasive than collecting samples with Salivette® Cortisol. The sampling method established should allow for regular tests and be easy and well accepted by patients, while providing comparable results.

Risks and methods related to the medical procedure

There are some risks involved in the nasopharyngeal mucosa sampling procedure using swabs, such as mild bleeding of the nasal mucosa, pharyngeal pain after the sample is taken, sneezing, cough, and gag reflex when taking the pharyngeal mucosa sample. There are no known risks for patients related to the Salivette® Cortisol device, and even accidental ingestion of the device has not led to any adverse reactions being reported, as it is made of inert material and does not contain any active or reactive substances.

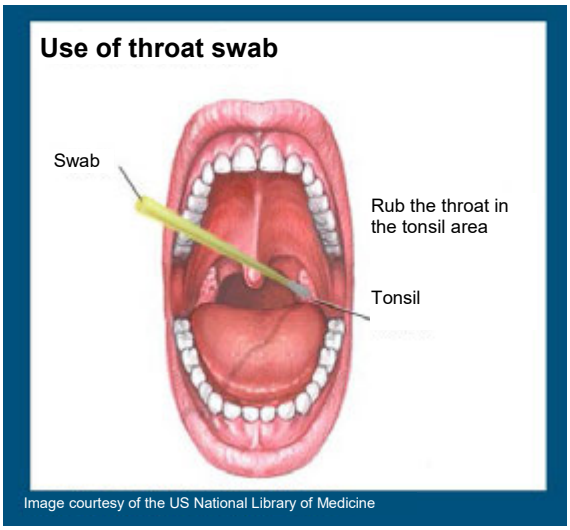
Sample taking with swab/Salivette® Cortisol

SARS-CoV-2 sampling:

The nasopharyngeal mucosa swab samples and those taken with the Salivette® Cortisol device will be collected in line with the SARS-CoV-2 biological sample collection standards in place at the Research Site.

Sampling

1. Only the health care staff and the individual undergoing the test should be in the room when the test is performed.
2. All of the non-aerosol generating procedures (documentation, interviews, blood sampling) must be performed first. The aerosol generating procedures (collection of oropharyngeal swab samples and saliva samples) must be the final activity performed.
3. It is likely that the collection of oropharyngeal swab samples will cause a cough
4. All samples must be identified with a unique code, using a permanent marker or a label
5. The identities of all samples must be listed separately, without including additional personal data of the patients alongside the samples
6. Collection of oropharyngeal swab samples
    1. Use a tongue depressor to prevent the tongue from interfering with the sample collection
    2. Insert the swab into the mouth
    3. Rub the swab on the tonsillar pillars and the posterior oropharynx, avoiding contact with the tongue, teeth and gums.

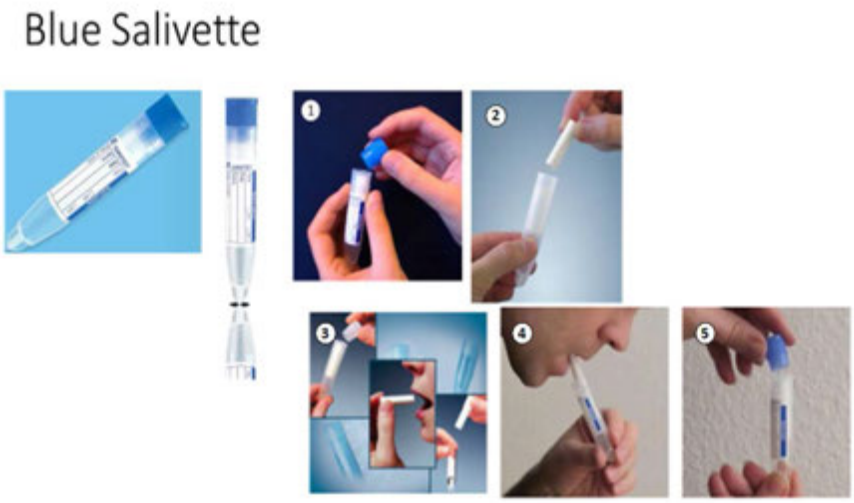


    4. Place the swab in the sterile viral transport tube and remove the applicator. Ensure that the cap is tightly sealed.

    5. Transfer the closed tube into a biohazard transportation bag and store the sample in a refrigerator (2-8°C) or freeze the tubes (< 20°C).

7. Saliva (Salivette® Cortisol)

1. Instructions for use



…and replace the cap (see Fig. 5).

Sample transportation

The biological samples obtained from the participant will be processed at a central laboratory in Germany, as described in section 2.1.4 TEST METHODS, and transported from Mexico in cold chain to ensure their stability. Sample transportation will be carried out in line with IATA specifications as category B biological samples.

1. Classification: UN3373 Biological Substance, Category B
2. Correct name for shipping: **UN3373 Biological Substance, Category B**
3. Packaging for international transportation: Samples must be packaged, shipped and transported in line with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation: packing instruction 6504.
4. Swab sample tubes to be sent between 4 and 8 degrees Celsius or in dry ice, as indicated by the carrier depending on the transportation time. (see section 2.1.6 Storage and transportation conditions – Mexico to Germany).
5. Each shipment must contain a full list of every sample's identity.
6. To ensure the traceability of the shipments, all of these will be organised by the receiving [redacted] in [redacted] using the World Courier service.

2. OBJECTIVES AND GOALS

2.1 MAIN OBJECTIVES AND PRIMARY GOALS

2.1.1 Main objectives

The main objective of the tests is to establish saliva collection with Salivette® Cortisol.

For PCR:

Real-time RT-PCR ([redacted])	+/-
Real-time RT-PCR [redacted]	+/-

2.1.2 Main diagnosis for admission

Suspected or confirmed cases of SARS-CoV-2 infections (including COVID-19), confirmed with PCR within the past five days.

2.1.3 Inclusion criteria

Only participants who meet the following criteria will be included in the programme:

1. Informed consent form signed and dated before any samples have been taken.

2. Suspected or confirmed SARS-CoV-2 infection by PCR within the past five days.

3. Male and/or female patients aged 18 years and over.

2.1.4 Withdrawal of consent or decision by BI to discontinue the test programme

Participants may withdraw their consent to participate at any time, without having to justify their decision.

2.2 TEST METHODS

The *in vitro* diagnostics have CE marking, are commercially available and are established for diagnostic purposes at the laboratories indicated. They were selected based on their reported sensitivity and specificity criteria, as well as their availability on the market. The laboratory [redacted] was chosen due to being a professional, certified laboratory which is highly experienced in serology methods and with experience in COVID-19 diagnostics since the start of the SARS-CoV-2 pandemic.

2.2.1 Identity of the *in vitro* diagnosis at BI

The test product characteristics are shown below:

Name:	**Kylt® for detection/confirmation of SARS-CoV-2**
Method:	Real-time RT-PCR
Source:	[redacted]
Diagnosis site:	
Sample:	Swab (throat) and Salivette® Cortisol
Duration:	4-6 hours
Name:	**cobas® SARS-CoV-2 (PCR) test**
Method:	Automated real-time RT-PCR for cobas® systems
Source:	[redacted]
Diagnosis site:	
Sample:	Swab (throat)
Duration:	3 hours
Name:	**TaqPath™ COVID-19 CE-IVD RT-PCR kit**
Method:	Multiplex RT-PCR
Source:	[redacted]
Diagnosis site:	
Sample:	Salivette® Cortisol
Duration:	4-6 hours

Real-time PCR detection of SARS-CoV-2

For the qualitative detection of SARS-CoV-2, a single-step RT-PCR method based on specific kits for SARS-CoV-2 or kits which include a primer and a probe (RT-PCR assay).

RT-PCR assays are suitable for implementing a multiplex RT-PCR focus in real time: in the case of a reaction, the RNA target sequences of the gene(s) of interest (e.g., SARS-CoV-2), and an endogenous control (e.g., β-actin) or an IPC (e.g., RNA phage control), are reversely transcribed and amplified in parallel with the corresponding pairs of primers in the polymerase chain reaction. The amplified target gene fragments are detected using probes marked with fluorescence during the real-time PCR reaction. In order to monitor each analysis run, general controls will be provided for performing the run (e.g. positive control and negative control of synthesis).

The specific probes for the gene(s) of interest and the internal control(s) (endogenous or loaded) are each marked with different fluorescent dyes (e.g. FAM and HEX), and their fluorescence emitted is optically measured separately with the real-time PCR thermocycler. Through the individual analyses in one reaction vessel per sample and the positive and negative controls per run, the status of a sample can be evaluated in terms of the gene(s) of interest **[Corman et al. 2020]**.

2.2.2 Storage and transportation conditions – Mexico to Germany

The test supplies will be kept in their original packaging and in a secure storage area with restricted access, in line with the recommended storage conditions (labelled). It is recommended to transport and store probes such as swabs and Salivette® Cortisol at 4-8°C.

For storage times of longer than 48 hours prior to shipment of swab and Salivette® Cortisol samples for storage in Germany, transportation at temperatures < 20°C in dry ice is recommended.

Packaging of all shipments must comply with the international regulations for transporting dangerous goods (UN3373 for dry ice).

2.2.3 Responsibility for the *in vitro* diagnosis

Only the documented authorised staff may receive and dispense *in vitro* diagnostic tests. These tests must be performed as stated in the product instructions or in the test protocol.

The occupational health care team must keep records of the diagnostic tests entering the test site, the site's inventory, use with each participant and the disposal of compromised devices.

All compromised devices will be disposed of locally by the test site. The receipt, use and disposal of the devices must be duly documented. Any discrepancies must be reported.

2.3 REFERENCE MEASURES

2.3.1 Restrictions

There are no dietary or lifestyle restrictions in place. However, a condition for taking biological samples is that no food or medicinal products may been consumed for a period of at least 30 minutes before the procedure to prevent saliva contamination.

2.3.2 Handling of swabs and saliva samples

The throat swab sample collected arrives in a transport medium. The swabs are washed within the transport media, by shaking the tubes for 10 minutes at approximately 500 rpm, in an orbital shaker at room temperature. The sample can be stored for up to 7 days in the transport medium, at +4°C. The saliva samples collected with Salivette® Cortisol are packaged in the corresponding primary container.

3. SAMPLE SIZE

There is no calculated sample size but rather a general attempt to directly compare swab samples versus saliva samples collected with Salivette® Cortisol in a participant, with a minimum of 20 to 80 participants.

4. ADVERSE EVENT REPORTING

Although this study is based on recently collected data, adverse event (AE) collection is not required. There is no Boehringer Ingelheim medicinal product intended or marketed for this disease. The investigator is encouraged to report all adverse events related to any product marketed by Boehringer Ingelheim, in accordance with the local regulatory requirements for spontaneous AE reporting at the investigator's discretion using the locally established means and AE report forms. The term AE includes the exposure to medicinal products during pregnancy and, regardless of whether or not an AE occurs, any abuse, off-label use, misuse, medication error, occupational exposure, lack of efficacy and unexpected benefit.

Other reports. As any reportable adverse events involving medical devices, technical complaints regarding the product and device deficiencies could cause harm to patients during clinical studies related to Salivette® Cortisol/swabs, the investigator will remain responsible for managing all activities, including reporting the medical device in line with the local regulatory requirements on reporting medical devices.

According to its definition, a reportable adverse event involving a medical device means any unfavourable medical event, disease or injury, or any unfavourable clinical sign, including abnormal laboratory findings, in subjects, users or other individuals, in the context of a clinical study, regardless of whether or not it is related to the investigational device, and which meets the criteria for a reportable event as set forth in the applicable laws and regulations.

5. REFERENCES

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