Clinical Performance of Dräger Antigen Test SARS-CoV-2

Clinical Performance Study Protocol

PROTOCOL AGREEMENT & SIGNATURES

Principal Investigator

I have read and understood the protocol below. In my capacity as **Principal Investigator**, my duties include making sure of the safety of the study participants enrolled by supervising them and providing **Dräger (Sponsor)** with complete and timely information. This information will be provided as outlined in this study protocol. All the information relating to this study will be held in strict confidence and these confidentiality requirements apply to all staff at this study site or involved with this study. I agree to maintain the procedures required to perform this study in accordance with Good Clinical Practice principles and to abide by the terms of this protocol.

11.02.2021

Principle Investigator Signature *Date*

Dr. Daniel Zickler

Name and Title (Print)

Sponsor

The signatories agree to fully comply with the Clinical Performance Study Protocol, all applicable national regulatory requirements for medical devices/IVD, the international standards of Good Clinical Practice (ICH-GCP) and the ethical principles of Declaration of Helsinki.

11.02.2021

Sponsor's Representative Signature *Date*

Dr. Rainer Poltnis

Name and Title (Print)

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PROTOCOL SYNOPSIS

STUDY TITLE	Dräger Antigen Test SARS-CoV-2 Clinical Performance Study
SPONSOR	Drägerwerk AG & Co. KGaA, Lübeck, Germany
PROJECT LEAD	Dr. Rainer Polzius
NUMBER OF SITE	20-GER-01
RATIONALE	The Dräger Antigen Test SARS-CoV-2 is a rapid lateral flow immunoassay for the qualitative detection of SARS-CoV-2 nucleoprotein directly from minimally invasive nasal swabs directly at the point of care. The coronavirus SARS-CoV-2 can cause a mild to severe respiratory illness, i.e. COVID-19, and has spread globally. Already existing rapid tests are mostly based on invasive nasopharyngeal sampling and involve cumbersome sample extraction procedures requiring well trained users. Given the simple and less invasive sampling methodology of the Dräger rapid test, it is worthwhile to execute the proposed study as no harm is expected for the participants and a rapid and easy test is to be gained.
STUDY DESIGN	Prospective, monocentric, clinical performance evaluation study for an IVD
PRIMARY OBJECTIVE	Determine general sensitivity and specificity of the Dräger Antigen Test SARS-CoV-2 in symptomatic participants in comparison to the reference method RT-PCR (at a threshold of ≥ 10^6 RNA copies/mL for positive PCR results)
SECONDARY OBJECTIVE(S)	• Sensitivity and specificity of the test under evaluation in relation to the ct value of the reference test • Sensitivity and specificity of the test under evaluation in relation to the time since symptom onset • Specificity of the test under evaluation in at least 100 asymptomatic participants • Frequency of nosebleed or unbearable pain during or immediately after specimen collection
INTERVENTIONS	No therapeutical interventions, no randomization
NUMBER OF PARTICIPANTS	approximately 850
PROCEDURE	Female and male adults with a pre-existing need for SARS-CoV-2 testing undergo screening for study participation suitability at the study site. Two separate sets of participants are included: persons with COVID-19 symptoms (symptomatic group) and persons who might have been exposed to SARS-CoV-2 but show no symptoms (asymptomatic group). Participants are required to meet all inclusion criteria and none of the exclusion criteria. All participants are tested with the Dräger Antigen Test SARS-CoV-2. RT-PCR results for pharyngeal swabs from the same participants serve as reference. This test is not initiated on the Sponsor's behalf; rather the Sponsor asks the participants to allow the PI to share pseudonymized PCR results as reference to compare to the results generated by the investigational product. All test results are collected for performance analysis of the Dräger Antigen Test SARS-CoV-2. Criteria for early study termination have been defined to avoid unnecessary strain for participants.

<table>
<tr><td>PARTICIPANT SELECTION CRITERIA</td><td>
<table>
<tr><th colspan="2"></th><th>Inclusion</th><th>Exclusion</th></tr>
<tr><td colspan="2">General</td><td>• ≥ 18 y old
• Written informed consent (ability & execution)
• Pre-existing need for SARS-CoV-2 testing, i.e.
o COVID-19 symptoms
o Known or suspected exposure to SARS-CoV-2
o Screening</td><td>• < 18 y old
• Pregnant or breast-feeding women
• Unable to provide informed consent
• Bleeding disorder
• Application of nasal spray prior to testing on the day of testing
• Involuntarily held in an institution
• Hospitalization/ inpatient</td></tr>
<tr><td rowspan="2">Specific</td><td>Asymptomatic</td><td>• Asymptomatic during the previous 14 days</td><td>• COVID-19 symptoms during the previous 14 days</td></tr>
<tr><td>Symptomatic</td><td>• COVID-19 symptom(s) present on the day of testing
• Symptoms started 0-7 days prior to testing day</td><td>• Symptom(s) started more than 7 days prior to testing day</td></tr>
</table>
</td></tr>
<tr><td>PRODUCT UNDER INVESTIGATION</td><td><u>Dräger Antigen Test SARS-CoV-2</u>
The investigational product is comprised of a sample collector and a test cassette. The sample collector retrieves the specimen from the anterior nose, while the test cassette analyzes the sample for the presence of SARS-CoV-2 nucleoprotein.</td></tr>
<tr><td>COMPARATOR</td><td><u>RT-PCR specific for coronavirus SARS-CoV-2</u>
Pharyngeal swabs are collected using the Cobas PCR Media Uni Swab Sample Kit (Roche). Samples are analyzed using the PCR-Kit Cobas SARS-CoV-2 (Roche). For scoring the PCR result negative or positive, a cycle threshold (ct) value is used. Unless otherwise indicated, this study scores a PCR result with a viral concentration of ≥ 10^6 RNA copies/mL as positive and < 10^6 copies/mL as negative. For data analysis, the corresponding ct value is provided based on PCR system calibration.</td></tr>
<tr><td colspan="2">DURATION OF PARTICIPATION AND STUDY DURATION</td></tr>
<tr><td>SCREENING</td><td>Study staff identifies eligible patients over the course of a month. If needed, this period can be extended.</td></tr>
<tr><td>TREATMENT</td><td>Specimens for the Dräger test and comparator method are collected during the same one patient visit.</td></tr>
<tr><td>FOLLOW-UP</td><td>None needed.</td></tr>
<tr><td>OVERALL DURATION</td><td>January – March 2021, active phase 1 month</td></tr>
</table>

1. INTRODUCTION

Coronaviruses are a large family of viruses which may cause illness in animals or humans (Masters, 2006; Weiss & Leibowitz, 2011). SARS-CoV-2 is an enveloped, single-stranded RNA virus of the β genus (Hasöksüz et al., 2020). The virus can cause a mild to severe respiratory illness named COVID-19 and has spread globally.

Several rapid tests have been developed based on immunochemical detection of viral nucleoprotein. The advantage of faster time to result compared to PCR testing and therefore earlier initiation of contact tracing is a great added benefit of rapid antigen tests (van Beek et al., preprint). Most of these tests are executed at the point of care by experienced users and allow for detection of an infection with SARS-CoV-2 in patients within the first 5 to 7 days since symptom onset.

Examples of existing rapid antigen tests:

- BinaxNOWTM COVID-19 Ag CARD (Abbott): *https://www.fda.gov/media/141570/download*
- Veritor™ System (Becton, Dickinson): *https://www.fda.gov/media/139755/download*
- Standard Q Covid-19 Ag Test (SD Biosensor): *https://bestbion.com/wp-content/uploads/2020/07/bestbiondx_GA_standardq_COVID-19-IgM-IgG-Duo_Ag20200713.pdf*
- Biocredit Covid-19 Ag Test (Biocredit): *https://www.biovendor.com/file/13419/1-2%20IFU_COVID-19%20Ag%20RapigenWatermarked.pdf?version=202005131414*

The above-mentioned rapid tests are mostly based on an invasive nasopharyngeal sampling procedure and involve cumbersome sample extraction procedures requiring well-trained operators.

The Dräger Antigen Test SARS-CoV-2 is a rapid lateral flow immunoassay for the qualitative detection of SARS-CoV-2 nucleoprotein directly from less invasive nasal swabs, to be performed at the point of care. The test kit contains all components required to carry out a test detecting SARS-CoV-2 nucleoprotein.

Given the less invasive sampling procedure and the straight-forward, fast testing procedure of the Dräger Antigen Test SARS-CoV-2, it is worthwhile to execute the proposed study as there is no harm expected for participating patients (see p. 10 for details). This study is designed as a non-interventional study, i.e. none of the Dräger test results are used to justify any patient management decisions.

2. STUDY DESIGN

The study is designed as a prospective, monocentric, clinical performance evaluation study. It is designed to demonstrate suitability of the Dräger Antigen Test for SARS-CoV-2 nucleoprotein detection in clinical nasal specimens. Real-time reverse transcriptase polymerase chain reaction (RT-PCR, here also short PCR) on specimens collected by pharyngeal swabs serves as a reference method for all participants of this study. In fact, participants are recruited from people presenting themselves for SARS-CoV-2 testing (pharyngeal swab and PCR) at designated testing locations of the study site. Each participant must meet all inclusion criteria of the respective group (i.e. symptomatic or asymptomatic). For details on the reference method and scoring (cut-off) refer to 4.3 and 6.2 below.

The goal of the study is to compare Dräger test results to the corresponding PCR data for overall sensitivity and specificity. Assuming a 20% PCR positive rate at the study site (prevalence), a 90% Dräger test sensitivity as the expected sensitivity, allowing room for

dropouts, and ensuring inclusion of at least 100 asymptomatic cases approx. 850 participants have to be enrolled (for details see case number estimation in 6).

Dräger provides approximately 900 Dräger test kits for this study to allow for practice runs and error. Should more testing become necessary after review of the half-way point or the endpoint, plans are made accordingly. When 425 data pairs of Dräger test and PCR results are available, the study reaches its half-way point. All data relevant for overall sensitivity and specificity are analyzed, and a brief overview of results serves as a preliminary summary at this point of time. Data relevant for secondary objectives are analyzed at the end of the study.

If people agree to participate, study staff records participants' information such as symptoms and time since symptom onset. For each study participant, the specimen for RT-PCR is collected first, then the Dräger test specimen. The participants self-sample the specimens using the Dräger sample collector closely supervised and guided by a qualified operator. Should the participant be compromised in some way such that self-sampling is not an option, the operator may collect the sample. The time of specimen collection for each test is recorded in the case report form (CRF) The Dräger test result is read after 15 min to 20 min, while the specimen for PCR is stored for further processing at a later time. Ideally, resulting data points are normally distributed along the age, gender, number of days with symptoms, and cycle threshold (ct) value or viral concentration spectrum.

3. STUDY OBJECTIVES

3.1 Primary Objectives

The primary objective of this study is to determine the levels of sensitivity and specificity of the Dräger Antigen Test SARS-CoV-2 based on direct nasal samples collected with the dedicated sample collector from symptomatic individuals requiring SARS-CoV-2 testing. The Dräger test result is compared to the reference method, that is RT-PCR. This study scores a PCR result of ≥ 10^6 RNA copies/mL (viral concentration) as positive (RKI, 2020a). The diagnostic laboratory analyzing the PCR samples for this study determines the corresponding cycle threshold (ct) value for their system.

3.2 Secondary Objectives

In addition to the primary objective the following secondary objectives are investigated:

Sensitivity and specificity (and CI for CL of 95%) of the Dräger Antigen Test SARS-CoV-2 are determined for

- both the symptomatic and asymptomatic groups,
- case groups sorted by ct value, and
- case groups sorted by days since symptom onset.

Goal is to explore the data and describe the relationship between the respective parameter and Dräger test performance.

The occurrence of lasting nosebleed or unbearable pain during or immediately after specimen collection is recorded for each participant. The frequency is monitored throughout the study. Its final value is presented in the study report.

4. STUDY PROCEDURE

4.1 Study Enrollment Procedures

Participants are presented for SARS-CoV-2 testing to medical staff. In order to determine whether a participant is eligible for this clinical study, candidates are asked to provide some information via questionnaire (see Case Report Form [CRF] for details) and consent to study participation. Participants are recruited from both symptomatic and asymptomatic people based on criteria detailed below.

4.2 Inclusion and Exclusion Criteria

If inclusion criteria are not met, the participant is excluded from the study.

Inclusion criteria of individuals

- Participants must require SARS-CoV-2 testing for the following reasons:
  - COVID-19 symptoms (Fever, cough and/or sore throat, fatigue/ general feeling of weakness, loss of sense of smell and/or taste, shortness of breath, muscle stiffness/ body aches, head cold/ running nose, etc.) on the day of testing, or
  - Known or suspected exposure to SARS-CoV-2 (contact persons etc.), or
  - Member of a group of high risk of exposure such as healthcare workers etc., or
  - Require screening for any other reason, e.g. doctor's orders, hygiene directive etc.
- Participant must be of legal age and must be able to understand the procedure and letter of consent.
- Patient provides written consent prior to enrollment.

Exclusion criteria of individuals

- Persons younger than 18 years old are excluded from the study.
- Pregnant or breastfeeding women are excluded from the study.
- Persons unable to provide written informed consent are excluded.
- Persons with bleeding disorder are excluded from the study as a precaution.
- Persons in institutions where they are (involuntarily) held due to an official order or a court order are excluded from the study.
- Hospitalized patients/inpatients are excluded.
- Application of nasal spray within 15 min prior to testing.
- Participants with symptoms on the day of testing are excluded, if any of their symptoms started more than 7 days prior to testing.
- Asymptomatic participants are excluded, if they experienced any symptoms in the 14 days prior to testing.

Inclusion of Dräger test samples/data

All data is presented and assessed in the study report. Only results with both valid Dräger test and valid PCR results are included in the data set for clinical performance assessment. A Dräger Antigen Test SARS-CoV-2 result is valid when:

- Pouch intact upon visual inspection,
- Desiccant bag present,
- Kit not expired,
- All components (sample collector, test cassette) present,
- Kit appears undamaged and ampoule is intact (no leaking apparent),
- Indicator ring is colored prior to testing,
- Results were read according to this study protocol.

4.3 Testing

Operators are trained in applying the Dräger test according to the instructions for use prior to collecting samples for this study. Training is acknowledged in writing. Patients who consent to participate in this study do not only provide a specimen for their planned PCR testing, but also for testing with the investigational product, the Dräger Antigen Test SARS-CoV-2.

Specimen collection

Trained clinic staff collect specimens for the Dräger test and for RT-PCR. For each sample, they follow instructions for use provided for this study or the site's standard operating procedure (SOP) for the Dräger test and RT-PCR, respectively. Samples are collected and treated as instructed, i.e. the Dräger test is run immediately and read after 15-20 min, while the sample for PCR is stored according to SOP until further processing.

Reading the Dräger Antigen Test SARS- CoV-2 results

The analytical detection limit of the Dräger Antigen Test SARS-CoV-2 is at 250 pg/test, i.e. with ≥ 250 pg of SARS-CoV-2 nucleoprotein present, a test line is visible. The following provides guidance on how to score the Däger test results. For additional detail refer to the Instructions for Use and the Investigator's Brochure.

Test strips are evaluated 15-20 min after starting the test. The viewing window of the test cassette holds the test strip (Fig. 1, below). One end of the strip is the designated control line area, while the other is the designated test line area. Both areas are indicated with C and T, respectively.

Figure 1. Viewing window with test strip and C and T line area labels; C = control line area, T = test line area.

Positive test result

If red control and test lines are present, the test result is positive. The intensity of the test lines can vary, meaning that very weak, partially colored or broken test lines are also scored as positive.

Negative test result

Test results are read 15 min after starting the test at the earliest and 20 min after starting the test at the latest. If – within this defined window of time - a control line is present while no test line is visible, the test result is negative.

Invalid

If neither a control nor a test line is present within 20 min of starting the test, the result is invalid.

PCR

Samples for PCR are processed in the diagnostics laboratory on site following routine procedures as described in Corman et al. (2020), Lindner et al. (in press), and Matheussen et al. (2020). In brief, the Roche Cobas SARS-CoV-2 assay (Pleasanton, CA United States) is performed.

RT-PCR specific for SARS-CoV-2 and the Cobas PCR assay specifically are considered a suitable reference method because of their e.g. high sensitivity, high specificity, low false-positive rate (Matheeussen et al. 2020, cobas SARS-CoV-2 IFU). As a quantitative assay it delivers information on the viral load for each sample, which allows test performance evaluation in relation to viral load in this study.

4.4 Collection of results

The study staff records patient and testing information (mostly by checking boxes) in the respective CRF:

- Age
- Gender
- Any COVID-19 symptoms? If yes, which and since when?

After Dräger Test specimen collection, the participants indicate the pain level of sample collection (none, light, strong, or unbearable), which is recorded in the CRF. Dräger Test results (positive, negative, or invalid) are entered on the corresponding CRF directly after testing. They are not shared with the participants and do not inform any steps regarding patient/participant management. If feasible, the information is also collected electronically.

PCR samples are processed within 72 h of sampling. PCR results are processed according to the Corona Test Center's regular procedures. They include the information of patients and authorities where applicable. If feasible, the information is also collected electronically.

4.5 Reporting

The PI is responsible for making the following reports available to the Sponsor.

Table 1. Overview

Report	Content
Daily Update	• No. of participants enrolled • Dräger test results per participant • Information regarding pain and nosebleed • PCR result per participant; as many results as are available at the time of reporting [1]
Weekly Summary	• Summary of daily data • Symptoms; days since testing (counts for 0-7 days and overall) • Ct value (or viral concentration) • Consent status per patient • AE summary
Half-way Report	• Due after 425 test result pairs were collected • Content as in Weekly Summary
Final Report	Compilation and review of all

1 – PCR results are provided with a lag time of 1-3 days compared to Dräger test results.

5. DEVIATIONS, ADVERSE EVENTS, AND SAFETY

5.1 Deviations

Deviations are defined as divergences from the Clinical Performance Study Protocol and must be reported and explained. The CRF is the preferred place to indicate a one-time deviation from the standard procedure. Should general (systemic and/or repeating) issues occur and have been identified as such, the PI is to inform the Sponsor's representative immediately. For an overview on documentation and notification requirements see below (p. 13).

5.2 Adverse events

The term adverse event (AE) describes events related to study activities for the performance evaluation of medical devices. It refers to undesirable, negative events such as injuries or diseases observed over the course of the study. General categories are presented and defined in Tab. 2 and Tab. 3, respectively, below.

Table 2. General categories of adverse events as defined by ISO 20916

Adverse events	Non-device-related [1] - Sampling procedure causes direct harm to participant -	Device-related - Device causes direct harm to user or another person -	
Non-serious	Adverse event	Adverse device effect	
Serious	Serious adverse event	Serious Adverse device effect	
		Anticipated	Unanticipated

1 Note: The Dräger Test kit includes the sampling device, i.e. the sample collector. That means that the sampling – unlike other IVD devices that may require a swab or blood draw - is part of the device-related procedure.

Table 3. Definition of adverse events categories. Definitions apply to studies subject to approval as well as studies with exemption from approval.

AE Category	Definition
Adverse Event, AE	Any untoward medical occurrence, unintended disease or injury in participants, operators or other persons
Adverse Device Effect, ADE	Adverse event related to the use of the Dräger test kit resulting from - insufficient or inadequate o instructions for use, o operation, or - malfunction
Serious Adverse Event, SAE	adverse event (study-related, but not necessarily device -related) that led to any of the following - death, - serious deterioration of health of the participant, operator or other persons including but not limited to life-threatening illness or injury, - fetal distress, fetal death or congenital abnormality or birth defect.
Serious Adverse Device Effect, SADE	Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event (see above)
Anticipated Serious Adverse Device Effect, ASADE	Effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report [1]
Unanticpiated Serious Adverse Device Effect, USADE	Serious adverse device effect which is by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report

1 See Tab. 4 and Risk Analysis Report for details specific for this study.

Overall, anticipated adverse events have been mitigated based on the current risk assessment (see Tab. 4 below).

Moreover, in an in-house study at Dräger with 46 individuals who were asked to score the experience of specimen collection with the Dräger sample collector as pleasant, neutral, non-pleasant, painful or severely injuring, most participants described it as neutral (Reference 11.5, Report on Usability of Sampling Unit). Two participants scored the sampling as non-pleasant, while nobody reported it as painful or experienced severe injuries. In 1 out of 46 individuals traces of blood were discovered on the sample collector; yet, this individual did not suffer from persistent bleeding after the sampling procedure.

Despite the fact that the nasal sample collection is less common than e.g. nasopharyngeal sampling, the sample collector itself has been used successfully for > 5 years in commercial test kits for saliva collection at the buccal mucosa for abusive drugs testing (Dräger DrugCheck 3000). More than 600000 tests have been sold so far and no complaint has been received with respect to safety issues in this time period. Biocompatibility, cytotoxicity, irritation and bioburden testing attested the sample collector to be safe to use (Reference 11.6, Biocompatibility Synopsis.

Table 4. Anticipated adverse device effects and their respective mitigation for the Dräger test kit

Possible adverse event	Fre-quency	Severity	Risk	Mitigation	Residual risk
Contaminated sample collector	Rare	Catastrophic (depending on the pathogen)	Infection and disease	Special care is taken during production; IFU instructs on hygienic use	None
Investigator/ Operator contact with specimen (nasal secretions) of the patient during sampling/ Investigator contact with specimen-buffer mix	Rare	Catastrophic (depending on the pathogen)	Infection and disease	Operator wears PPE. Buffer is not harmful. Usual cleaning and disinfection procedures are sufficient. If preferred, a zip loc bag is provided for use during this step.	None
Nosebleed < 15 min	Rare	Non-serious	Injury, pain	Observe the patient closely; if bleeding stops, continue the investigation. If it does not stop after 15 min, participant is transferred to emergency department for appropriate treatment and removed from the study. See also lasting nosebleed below.	None except potential time (< day) in the ER
Lasting (> 15 min) nosebleed requiring intervention	Rare	Non-serious for most patients, serious for patients with bleeding disorder	Blood loss	Exclude patients with bleeding disorder; study staff awareness; Stop of participation in the study for appropriate treatment	None except potential time (< day) in the ER

Pharyngeal swabs are the sampling method for the comparator. As these specimens are not collected exclusively for this study, rather this study uses the results of the current default COVID-19 screening at the site, the procedure is not included in this study's risk assessment.

5.3 Criteria for interruption or early termination of the study:

For the individual participant

If nosebleed occurs in a participant, specimen collection is interrupted until the nosebleed stops. If it stops in less than 15 min, the investigation can continue with the second nostril. An Adverse Device Effect (ADE) is reported in the CRF.

If the nosebleed does continue after 15 min of basic measures, the study investigation is stopped for this participant, and the participant is transferred to the emergency department to respond appropriately to stop the nosebleed. An Anticipated Serious Adverse Device Effect (ASADE) is reported in the CRF and the Sponsor is informed immediately.

If the patient experiences severe pain, discomfort or wants to stop the procedure, the investigation is stopped for this patient and an AE or ADE is reported depending on the evaluation of the study physician.

Participants have the right to withdraw from the study at any time for any reason without penalty or prejudice. The investigator also has the right to withdraw patients from the study if he/she feels it is in the best interest of the patient or if the patient is uncooperative.

For the study in general

(1) The study stops immediately, if and when approval of the study/exemption of approval by the Authorities or the Ethics Committee is withdrawn or retracted.
(2) The study is stopped early, if > 10% of participants experience lasting nosebleed (longer than 15 minutes) or unbearable pain. This assessment starts with the first 50 participants enrolled. See also 7. Monitoring below.
(3) The Sponsor reserves the right to discontinue the study at any time in its entirety e.g. if, in the sponsor's opinion, there is a significant safety concern, relevant technical problems with the device or an insufficient recruitment despite intensified efforts to screen potential participants. See also 7.4 Monitoring below.

5.4 Documentation requirements

All unexpected observations and/or adverse events must be reported and documented on the CRF by Operator and Principal Investigator (PI) (Tab. 5).

The PI has the responsibility to document and explain any deviations or adverse events, actions taken, and notify the sponsor represented by a designated Project Lead (refer to 9.1 Site and Sponsor Representatives for details).

5.5 Notification requirements

The table below describes the PI's responsibilities regarding Sponsor notifications.

Table 5. Event documentation and sponsor notification

Event	Document on CRF	Notify via E-mail	Notification Timing
Deviation	yes	Sponsor's Representative	Within two weeks
AE, ADE	yes	Sponsor's Representative	Within two weeks
SAE, SADE	yes	Sponsor's Representative	Immediately; at the latest within 24 h
ASADE, USADE	yes	Sponsor's Representative	Immediately; at the latest within 24 h

The Sponsor informs the Competent Authority in case of SAEs via e-mail to MPSAE@bfarm.de.

6. STATISTICAL AND ANALYTICAL CONSIDERATIONS

All cases are included in the overall analysis and evaluation of the study and in the resulting report.

In general, numbers of participants (eligible, enrolled, included in analysis), Dräger test and PCR results, invalid tests, dropouts at every level, deviations, AEs etc. are recorded, monitored throughout the study, and reported in the final study report. Performance data is presented in the context of these counts. Only cases for which both a valid Dräger test and a valid PCR result are available are included in the determination of performance parameters such as sensitivity and specificity (see below). In addition, the following information is required for a case to be included, i.e. a case is excluded should the following information not be documented:

- Participant ID
- Participant provided written informed consent.
- Information regarding any pain sensation
- Information regarding nosebleed
- Information regarding adverse event (on the level of yes or no)

6.1 Case number estimation

For the case number estimation for the primary endpoint the following assumptions are made:

Sensitivity:

Lindner et al. (in press) collected samples from the anterior nose and observed 27 of 29 SARS-CoV-2 PCR-positive samples (> 10^6 copies/mL) to also be rapid antigen test-positive (93.1% agreement). The case number estimation for the Dräger Antigen Test SARS-CoV-2 is based on a more conservative expectation of 90% sensitivity. This assumption is based on preliminary performance data of the test under evaluation and the threshold for PCR set to 10^6 copies/mL (refer to 6.2 for details).

The half width of the confidence interval (W) is pre-determined at 5%, as 90% ± 5% provides sufficient test reliability for a daily application in the clinical setting. A test sensitivity of 85% would still meet the PEI criterion for antigen tests (sensitivity > 80%; PEI, 2021).

Specificity:

Lindner et al. (in press) observed a rapid antigen test specificity of 99.6% (95% CL, CI 97.8-100.0%) in anterior nasal swabs. As a result, the investigational product is expected to be 99% specific. For diagnostic applications, a device should deliver a specificity of 99% with a precision of ± 1% in order to be of service, e.g. in the screening of symptomatic patient at times of high incidence. It also needs to be taken into account that the intended use of the investigational product is for screening; ultimate confirmation of the preliminary result remains for more sensitive PCR testing.

Separate calculations for case numbers for sensitivity and specificity of the test are performed following the approach by Hajian-Tilaki (2014). The following formulae are used:

$$N(SN) = z^2 \times \frac{(SN(1-SN))}{W^2 \times P}$$

$$N(SP) = z^2 \times \frac{(SP(1-SP))}{W^2 \times (1-P)}$$

with

- N = Case Number
- SN = Sensitivity
- SP = Specificity
- z = z-value corresponding to a confidence level of 95%
- P = Prevalence of disease in the test population. Prevalence is assumed to be at least 20% based on current data from the prospective study sites indicating an actual prevalence among all participants tested for SARS-CoV-2 of 25%.
- W = maximum marginal error (half width of 95% confidence interval) for sensitivity and specificity, respectively

The resulting case number estimation for sensitivity evaluation of the investigational product is 691. Assuming a 5% dropout rate, 726 participants have to be enrolled. For specificity evaluation, 500 participants (allowing for up to 5% dropouts) need to be enrolled. Since the prevalence of PCR-negative participants is assumed to be at 80%, more test negative cases (580) are expected to be enrolled in the study for the determination of sensitivity alone. As a

result, the cases needed for specificity assessment are covered by the case number estimated for sensitivity assessment. In explicit, 726 participants are enrolled to sufficiently power overall sensitivity and specificity evaluation of the test product.

Based on the current testing strategy (Federal Ministry of Health, Germany, Dec. 16, 2020) and according to communications with the study site (Dec. 2020), more symptomatic than asymptomatic persons present themselves for PCR testing at the site. In order to ensure a minimum of 100 asymptomatic cases to be included in the performance evaluation of the investigational product (PEI, 2021), an additional 105 participants are added to the total case number. This allows for a 5% dropout rate among asymptomatic participants, and it ensures performance evaluation of the test for asymptomatic people even if most or all cases enrolled for overall sensitivity and specificity evaluation belong to the symptomatic group.

As a result, the total case number estimate for this study adds up to 726 + 105 = 831.

6.2 RT-PCR, cycle thresholds and viral concentration

PCR as the gold standard for SARS-CoV-2 detection provides the benchmark for this performance evaluation study, i.e. a true representation of the actual SARS-CoV-2 state of the participant (positive or negative). Following RKI reasoning (RKI, 2020a), RT-PCR results are not only scored as absolute results of positive or negative, but also in the context of detected viral load per sample within the PCR positives. The RKI provides a threshold of 10^6 copies/mL beyond which the viral culturability potential seems neglectable (cell culture experiments as *in vitro* test to indicate infectiousness) (RKI, 2020b). Based on the PCR assay and cycler combination used for sample analysis, this viral concentration translates into a system-specific cycle threshold (ct) value for each sample, when the system is calibrated with reference standards (RKI, 2020a). This study uses the system-specific ct value corresponding to 10^6 RNA copies/mL to sort the PCR positives into either above or below this threshold.

Sensitivity and specificity of the Dräger test are determined based on true- and false-positive as well as true- and false-negative counts determined by comparing the Dräger test results to PCR results scored as follows:

- PCR-negative: negative PCR and positive PCR with viral concentration < 10^6 copies/mL, and
- PCR-positive: positive PCR with viral concentration ≥ 10^6 copies/mL.

Based on RKI information (RKI, 2020a), this ct cut-off value is expected to be at approximately 30 cycles and is determined by the diagnostic laboratory analyzing the PCR samples for this study.

6.3 Assessment of primary objective

Dräger test and PCR results for all participants are recorded. All valid data pairs (valid Dräger test result, valid PCR result, complete records as defined on pp. 13) from the symptomatic group feed into sensitivity and specificity evaluation of the Dräger test. A contingency table is used to present sensitivity, specificity, and associated confidence intervals (CI at a confidence level of 95%).

Table 7. Assessment of results.

Dräger Antigen Test SARS-CoV-2 result	**Reference method RT-PCR result**	
	Positive	**Negative**
Positive	True positive (TP)	False positive (FP)
Negative	False negative (FN)	True negative (TN)

Key parameters to be determined:

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

$$\text{Specificity} = \frac{\text{TN}}{\text{FP} + \text{TN}} \times 100$$

6.4 Assessment of secondary objectives

Secondary endpoints are of explorative nature and descriptive statistics are used for evaluation (mean, median, minimum, maximum, frequencies, standard deviation, 95% CL confidence limits).

All valid data pairs (valid Dräger test result, valid PCR result, complete records) feed into sensitivity and specificity evaluation of the Dräger test. Sensitivity and specificity including lower and upper confidence limits for the test under evaluation are calculated for different cut-offs of the ct value of the PCR test in addition to the predefined ct value for the primary endpoint (currently defined by a viral concentration of 10^6 RNA copies/mL and to be calibrated to ct). Prerequisite for inclusion in this explorative evaluation is that the case record is not only complete as defined above, but also provides the information required to allow sorting into groups according to e.g. ct values.

Sensitivity and specificity including lower and upper confidence limits for the test under evaluation are calculated for participants grouped by

- ct value (as described above),
- time since symptoms onset (using the general cut-off ct value of the PCR test used for the primary endpoint evaluation; on symptomatic group only),
- symptom status (symptomatic vs asymptomatic).

In addition to sensitivity and specificity centric explorative evaluation, the frequency of lasting nosebleeds or unbearable pain in participants is tracked. The primary information is captured in the CRF. Once the 50 participants have been enrolled the fraction of participants reporting such experiences is determined daily.

7. DATA QUALITY ASSURANCE

7.1 Training of study staff

Staff receives hands-on training for the Dräger Antigen Test for SARS-CoV-2 prior to collecting study specimens. This training is documented on the Operator Training Form (see Appendices below).

Red-green visual impairments disqualify staff as operators for this trial.

7.2 Case Report Form (CRF)

For each participant enrolled in the study, a case report form collects all study-relevant information. The corresponding patient file is archived at the study site. These two records are linked only by participant ID, as the clear name of the patient is not recorded on the case report form.

The operator documents the respective steps of the procedure and other case information in the CRF. Operator and Principal Investigator confirm these records are complete. Corrections are made such that the original entry stays legible. The new entry is dated and signed, too.

7.3 Archiving/Record retention

Data generated are documented, recorded, reported and archived in conformity with the CPSP and national standards. Records (Investigator Site File and Trial Master File) are retained for at least 10 years after completion or suspension of the trial. The Sponsor keeps a part of the documentation relating to the trial for as long as the product is authorized.

7.4 Monitoring

Monitor

The Sponsor defines a representative as Monitor of the Study prior to study start. The Monitor is the primary contact for the clinical study site and all questions related to the study. In addition, the Monitor checks in with the site and may request information or documentation pertaining to the study as she/he sees fit (prior to and during the trial).

Selected original documentation and database entries are compared by the Sponsor as a cross-check.

Sponsor

The Sponsor receives regular updates from the site on study progress, adverse events and preliminary data. This information is used to determine the fraction of all participants reporting pain or/and nosebleed as a result of specimen collection (or the frequency of pain and nosebleeds). When reaching 50 enrolled participants, the counts are tracked daily using the PI's daily updates and any potential Adverse Event notifications. Should at any point of time > 10% of participants report unbearable pain or lasting nosebleed the study stops.

The Sponsor's Decision Board - in its role as the complaint handling entity for clinical studies - evaluates, e.g. every SAE that may occur during the study and defines next steps.

At half-way point of the study the Sponsor's Decision Board reviews an overview of study-related information, e.g. device performance, AE summary, enrollment. The goal is to evaluate overall study progress and current safety and performance status of the investigational product. The board may take the decision to interrupt the study or terminate it early. Refer to 5.3 for selected scenarios.

7.5 Audits

Audits can be scheduled as needed. Currently, no inspections are planned during the course of this study.

7.6 Privacy

Data collected during this study includes personal information. Patient-related information including the clear name is managed, stored and archived only at the study site. The Sponsor only receives study-relevant information as collected in the CRF (which is identified by participant ID, not the patient name, i.e. pseudonymized).

Only dedicated study staff enter into and access study data records, i.e. access is restricted to authorized personnel, all of which abide current legislation on privacy.

Should a study participant decide to retract from this study, any existing entries would be deleted. Any personal information associated with this study would be destroyed after up to ten years, unless defined otherwise by legal or in-house requirements.

8. ETHICS AND REGULATORY REQUIREMENTS

All parties involved in this trial execute the study according to applicable laws and regulations and under consideration of the WMA Declaration of Helsinki.

The Ethics Committee of the site reviews the study protocol and accompanying documents. Suggestions by the Ethics Committee are taken into account such that the study can be approved and conducted. The study cannot be conducted should the Ethics Committee disapprove of parts of or the whole study protocol. The committee is also informed about any safety issues that might occur during the study as well as the closing of the study.

9. STUDY TEAM

9.1 Site and sponsor representatives

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Clinical and scientific experience of principal investigator(s) is demonstrated in the attached CVs and/or certificates.

9.2 Funding

The study is funded by Dräger.

10. PUBLICATION

A Clinical Performance Study Report summarizes the events and outcome of the study. Key results are included in the Instructions for Use to be provided to kit users upon purchase or request after product release. The PI may publish the outcome of this study as a scientific publication after product release. Details are addressed in a separate contract.

11. REFERENCES

11.1 Instructions for Use Dräger Antigen Test SARS-CoV-2

11.2 Risk Management Report Antigen Test SARS-CoV-2

11.3 Clinical Study Risk Evaluation

11.4 ISO 20916:2019

11.5 Report on Usability of Sampling Unit

11.6 Biocompatibility Synopsis

11.7 Instruction for Use Roche Cobas SARS-CoV-2

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