Study protocol

COVAG

COVID 19 **A**nti**g**en Study

Sponsor:
Synlab Holding Deutschland GmbH
Gubener Str. 39, 86156 Augsburg

SYNLAB Analytics & Services Germany GmbH Pharma Berlin
Turmstr. 21
10559 Berlin

Authors:
Sabrina Strauch
Carolin Kurth
Camovis GmbH
Wilmersdorfer Straße 79
10629 Berlin

Winfried März
SYLAB Academy
Synlab Holding Deutschland GmbH
Gubener Straße 39, 86156 Augsburg
P5,7, 68161 Mannheim

Version number: 1.2
Date: 05.01.2021

Responsibilities

Medical Director of the NIS

Dr. med. Martin Roskos
CMO (Chief Medical Officer)
SYNLAB Holding Deutschland GmbH
Weiden GmbH
Gubener Straße 39
86156 Augsburg
Tel.: +49 3641 5074 20
Fax: +49 3641 7074 11

MVZ Gera/SYNLAB Jena Oncoscreen
Straße des Friedens 122
07548 Gera
Tel.: +49 3655 5220 5201
Fax: +49 3655 5220 5202

Email: martin.roskos@synlab.com

Project Leader

Prof. Dr. Winfried März
Director SYNLAB Academy
SYNLAB Holding Deutschland GmbH
SYNLAB Akademie
Gubener Strasse 39
86156 Augsburg / Germany
Tel.: +49 621 4317 9432
Fax: +49 6221 793 144
Email: winfried.maerz@synlab.com

Steering group /NIS

Dr. Ulrike Hauser
Leitung SYNLAB-Schwerpunktlabor Infektionsserologie
SYNLAB MVZ Augsburg GmbH
Gubener Straße 39
86156 Augsburg
Tel.: +49 821 22780 0
Fax: +49 821 525295
Email: ulrike.hauser@synlab.com

Dr. Caren Mutschmann
Vice President Pharma
SYNLAB Analytics & Services Germany GmbH
Pharma Berlin
Turmstraße 21
10559 Berlin
Tel.: +49 30 396085 13
Email: caren.mutschmann@synlab.com

Dr. med. Christian Mayer
Bereichsleiter
SYNLAB Holding Deutschland GmbH
Zentralbereich
Gubener Straße 39
86156 Augsburg
Tel.: +49 821 52157 0
Fax: +49 821 52157 125
Email: christian.mayer@synlab.com

Local project responsibility

Study Site: Stuttgart Cannstadter Wasen / Leinfelden-Echterdingen

Dr. med. Hans-Jörg Wertenauer
Email: hj.wertenauer@aerzteschillerplatz.de
Corona-Testzentrums Cannstatter Wasen
Mercedesstr.50
70372 Stuttgart
Fax: +49 (0) 711 25241020

Prof. Dr. med. Eberhard Wieland, FEBLM/MB, EurSpLM
SYNLAB MVZ Leinfelden-Echterdingen GmbH
Email: eberhard.wieland@synlab.com
Nikolaus-Otto-Straße 6
70771 Leinfelden-Echterdingen / Germany
Tel: +49 711 9033-240
Fax; +49 711 9033-131

Statistician

Dr. Alexander Dressel
Dr. Dressel Consulting
Am Exerzierplatz 23
68167 Mannheim
Tel.: 0621-48247388
Fax: 0621-48247389
Email: dressel@dresselconsulting.de

1. Background

Coronavirus disease 2019 (COVID-19) has quickly spread worldwide from Wuhan (China) since December 2019. Reliable diagnostic technologies are pivotal to the fight against COVID-19. While real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) based testing of respiratory specimens remains the gold standard for COVID-19 diagnostics, several commercial assays for antibodies against (SARS-CoV-2) have emerged during the previous months (Stocking et al., 2020).

Measuring the presence or absence of viral proteins (antigens), antigen tests may provide results diagnosing an active SARS-CoV-2 infection much faster than RT-PCR or equivalent tests. It has been argued that antigen tests may have a lower sensitivity than RT-PCR or equivalent RNA-based tests, and as a result they are much more likely to give false-negative results in individuals with lower amounts of virus. This may possibly make them unreliable in individuals with an asymptomatic course of the disease, in individuals in the first days of infection, and later than 5-7 days after onset of symptoms (SYNLAB press release, 22.09.2020).

2. Objective

The objective of this non-interventional study is to demonstrate that rapid antigen tests for SARS-CoV-2 (Abbott and Roche) provide fast results and perform equally well than RT-PCR, at least in symptomatic patients with high viral loads.

3. Study Design

The study will be performed in at the Corona Test Center Stuttgart Cannstadter Wasen operated by Dr. med. Hans-Jörg Wertenauer, Stuttgart, in close collaboration with the SYNLAB MVZ Leinfelden-Echterdingen GmbH. Approximately 2000 patients will be enrolled at after having signed the Informed Consent Form (ICF). Upon a scheduled study duration of eight weeks, we will be able to collect the required 2000 samples (50 patients per day).

One study visit is planned for data and blood collection (15,5 ml), antigen tests, RT-PCR, serological tests, basic laboratory tests to be communicated to participants, and ancillary blood tests (incriminated to be related to COVID-19 like inflammatory markers, and micronutrients and vitamins). These will be performed at the at the SYNLAB MVZ Leinfelden GmbH. Aliquots of the materials collected will be stored -20 degrees for control measurements and ancillary tests, maximally for one year after the last patient was enrolled into the study.

4. Rationale

In the past weeks, there has been much discussion about rapid antigen tests for SARS-CoV-2. The most recent generation of these tests, available from several different suppliers, are expected to perform well in patients with high viral loads. This is usually the case approximately 3-10 days after infection with the virus, when the person is contagious, and may be asymptomatic or in the first days of symptoms.
It is therefore hoped that rapid antigen tests can support diagnosis of COVID-19 in people who have the first symptoms. In the USA, some tests have received authorization for diagnostic testing in symptomatic individuals, within 5-7 days from the onset of symptoms (3).

5. Risks and benefits

Three nasopharyngeal swabs and one blood sample will be obtained from each of the participants. Antigen tests, RT-PCR and serological and ancillary laboratory tests are performed in this study.

Risks from nasopharyngeal swabs: The biggest risk is discomfort. Rarely - 1 in thousands — the person receiving the swab passes out from being super sensitive or gets a mild nosebleed.

Risks from blood draw: The risks of venipuncture include Minor bruising at the site where the blood is taken and hematoma (12 % approximately), with minor bruising being the most common reaction. Significant complications (defined as cellulitis, phlebitis, diaphoresis, hypotension, near syncope, syncope) are observed in approximately 3.5% of patients. The most frequent single significant complication is hypotension at a frequency occurred of approximately 2.6%. Syncope is seen in less than 1% of patients. Other serious local reactions such as cellulitis or phlebitis are very rare (Galena HJ, J. Fam. Pract. 1992 May;34(5):582-4). Blood sampling is therefore conducted in each of the study sites under the surveillance of a board licensed physician.

Benefits to public health: Point of care antigen testing is an integral component of the current national testing strategy serving for preventing of the spread of COVID 19 https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/3_Downloads/C/Coronavirus/Nationale_Teststrategie_kurz_041220.pdf

There is, however, significant uncertainty as to the reliability of antigen testing in comparison to the gold standard of testing, rt-PCR. To the authors´ knowledge a prospective, sufficiently sizeable and challengeable evaluation of antigen testing under

practice conditions has not been completed so far. The current study is intended to close this gap. Its results are urgently required and will have immediate impact on current recommendations for using antigen testing. In particular, it will demonstrate weaknesses and strengths of antigen testing and will allow to outweigh potential technical limitations of antigen testing compared to rt-PCR against the advantage of short-term availability of test results.

Further, the thorough recording of the clinical health status and the basic laboratory analysis performed in this study, it will be able to get further insights into clinical predictors for the susceptibility for COVID 19.

Benefits to the individual. Participants will benefit in two ways. Other than with rt-PCR, they will obtain the results of the antigen testing at once and on-site. In addition, offering two antigen tests simultaneously, the results of the point-of-care testing will be even more reliable than the result of a single test. Further, the participants are offered a free of charge basic laboratory health check including the following actionable health markers: basic blood cell count, C-reactive protein, HbA1c, cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, gamma-glutamyl-transferase, creatinine along with formula-derived creatinine, vitamin D.

We conclude that benefits of this study to the healthcare system which is faced with the life-threatening and to the individual study participants clearly outweigh the minor and manageable risks.

6. Population

We will include approximately 2000 consecutive male and female patients aged 18 years or above attending the Corona Test Center Stuttgart Cannstadter Wasen, in whom RT-PCR testing for SARS-CoV-2 is medically indicated or requested.

6.1 Inclusion Criteria

Subjects eligible for inclusion in this study must meet the following criteria:

1. Signed informed consent must be obtained prior to study participation. The patient must be capable of understanding the nature, significance and implication of the trial.
2. Age 18 years or more

6.2 Exclusion criteria

1. Lack of informed consent
2. Inability to understand the nature, significance and implication of the trial.
3. Severe clinical conditions requiring emergency hospitalization
4. Children and adolescents under the age of 18 years

7. Informed consent procedures

Eligible subjects may only be included in the study after providing IRB/IEC–approved informed consent. Informed consent must be obtained before performing any study-specific procedures (Appendix 2).

8. Assessment schedule and assessments

Subject Table 8-1 Assessments

Assessments	Visit 1
Informed Consent Form, Appendices 1 and 2	X
Collection of demographic data and relevant Medical History (Appendix 3)	X
SARS-CoV-2 Rapid Antigen Test (Roche), Appendices 8 and 9	X
PANBIO Covid-19 Ag Rapid Test (Abbott), Appendices 5, 6 and 7	X
RT-PCR	X
Serological test (blood sample, 15,5 ml) Ancillary testing	X X

8.1 Demographics, Medical history, sampling

Patient demographic data and other characteristic data to be collected on all patients include sex, age, weight, height, relevant medical history, vital signs and symptoms characteristic of COVID-19 including precise time specifications for onset of symptoms (Appendix 3).

No information about medication will be collected.

Three independent nasopharyngeal swabs will be obtained following standard procedures

To avoid any bias due to the sequence of taking the nasopharyngeal swabs, we will randomly allocate the patients to three sampling groups

Group 1: RT-PCR – Antigen Test Roche – Antigen Test Abbott
Group 2: Antigen Test Roche – Antigen Test Abbott - RT-PCR
Group 3: Antigen Test Abbott - RT-PCR - Antigen Test Roche

The assignment of patients to each of these groups will be clearly specified on the case record forms. There will be two forms for each patient:

Form 1: Routine analysis request sheet as used by the collection point for patient identification which will be labelled with a bar code carrying the study number of a patient

Form 2: Study specific form containing a copy of the bar code with the study number, initials of the patient and the year of birth.

8.2 SARS-CoV-2 Rapid Antigen Test (Roche)

The SARS-CoV-2 Rapid Antigen Test is a rapid point of care chromatographic immunoassay for the qualitative detection of specific antigens of SARS-CoV-2 present in the human nasopharynx. (Appendices 8 and 9)

8.3 PANBIO Covid-19 Ag Rapid Test (Abbott)

Panbio COVID-19 Ag Rapid Test Device is an *in vitro* diagnostic rapid test for the qualitative detection of SARS-CoV-2 antigen (Ag) in human nasopharyngeal swab specimens. (Appendices 5; 6 and 7)

8.4 RT-PCR

RT-PCR evaluates the presence or absence of the SARS-CoV-2 genome in samples from nasopharyngeal and oropharyngeal swabs and will be conducted according to the protocols established at the SYNLAB MVZ Leinfelden GmbH.

8.5 Serological and ancillary testing

Blood samples (10 ml heparin plasma and 5.5 ml EDTA plasma) will be collected to detect the presence of antibodies (specific immune response) against SARS-CoV-2 antigenic proteins (e.g. Roche, Abbott). Specifically, all participants will be offered to receive the results for the following analytes in acknowledgment of their participation in the study: blood cell count, C-reactive protein, HbA1c, cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, gamma-glutamyl-transferase, creatinine along with

formula-derived creatinine, vitamin D. Backup samples will be collected to determine ancillary parameters like inflammatory markers, and micronutrients and vitamins.

9. Observation Period

NIS schedule:

Start of the patient recruitment: February 1, 2021
End of the observation period: March 31, 2021
Final report: May 31, 2021

10. Data analysis and statistical methods

A database of the pseudonymized data and results will be generated. We hypothesize that each of the antigen tests has a sensitivity and specificity of 1.0 in relation to RT-PCR which will be considered as the reference test. A p value of less than 0.05 will be required to consider each of the two rapid tests equivalent to RT-PCR.

With the aid of *power calculations for chi-square tests* for two degrees of freedom, required type I error probability equal to 0.05, required (1-type II error probability) equal to 0.8 and required effect size equal to 0.1 (small effect size in the sense of (4)) we obtain a number of at least *N=964* observations for consecutive testing of incoming patients in order to fulfill our requirements on significance and power to answer the first research question. Because of the expected number of around 10% positive out of 1000 cases, the assumption of a chi-square distribution is permissible.

We will also address the question whether different pre-test probabilities will affect the sensitivity of the of the antigen tests. We expect two major indication groups with different pre-test probabilities contributing approximately 90 percent of the rt-PCR positive samples: a) participants admitted for rt-PCR testing by a physician b) participants seeking for rt-PCR testing upon recommendation my health care authorities/contact persons. A total of approximately 200 rt-PCR samples will be required to detect a statistically significant difference between these two groups at a type 1 error of 0.05 and a statistical power of 0.80. To answer this second question, we will extend the study to 2000 participants.

11. Ethics

The study protocol will be submitted to the Ethics Committee the Medical Faculty Mannheim, University of Heidelberg and the study will not begin until written permission is obtained. The study will be conducted in accordance with German law, EU Clinical Trial regulations and the Declaration of Helsinki on the ethical principles of clinical research.

11.1 Patient information and Informed Consent

The objective, nature and extent of the document must be explained in writing to every patient before he or she is included in the study (Appedix 1). The patient must not be included unless he or she has given written consent (Appendix 2). Patients will have the right to refuse to participate or to withdraw from the study for any reason and at any stage without affecting the quality or accessibility of their health care.

11.2 Data protection

The patient data collected for the study will be recorded in a pseudonymised form (Appendix 4), with a patient number that cannot be attributed to the identity of the patient. In the event of the study results being published, the personal data may only be used if the patient's anonymity is upheld. The patient's data is guaranteed to be always absolutely protected.

12. References

1 Stocking, C., Hauser, U., de Miguel, L., Suteu, G., Dressel, A., Soricelli, A., Roskos, M., Valor, S., Mutschmann, C. & März, W. 2020. Evaluation of five commercial serological assays for antibodies to SARS-CoV-2, submitted

2 Pressemitteilung SYNLAB. 22.09.2020

3 CDC 4.9.2020. Zwischenleitfaden für schnelle Antigentests für SARS-CoV-2

4 Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences. New York: Routledge, https://doi.org/10.4324/9780203771587

13. Appendices

1. Appendix 1_PARTICIPANT INFORMATION SHEET
2. Appendix 2_PARTICIPANT CONSENT FORM
3. Appendix 3_CLINICAL REPORT FORM
4. Appendix 4_PARTICIPANT IDENTIFICATION LOG
5. Appendix 5_120007007 v1 Panbio COVID-19 Ag Nasopharyngeal QRG EN
6. Appendix 6_120006841 v1 Panbio COVID-19 Ag IFU Multilingual Gl
7. Appendix 7_ 120006839 v1 Panbio COVID-19 Ag Sell Sheet EN Globa
8. Appendix 8_ 40338-2 OnePager SARS-CoV-2_A4_V2_low_KSC
9. Appendix 9_ SARS-CoV-2 Rapid Antigen Test MS_V1_A3