Protocol for generic testing of antigen test for SARS-CoV-2 in Denmark

Prepared and relevantly modified according to STARD 2015 and the SPIRIT 2013 statement for reporting of diagnostic accuracy studies and standard protocol items for clinical trials.

Administrative information

Title	Agreement of antigen tests on oral pharyngeal swabs or less invasive testing with RT-PCR, for detecting SARS-CoV-2 in adults: A prospective nationwide observational study
Trial registration	www.clinicaltrials.gov
Protocol version	May 19th, 2021; v2.4
Funding	Companies providing antigen tests, hereafter termed the providers. - The provider delivers 800 antigen-tests free-of-charge. - The provider will pay a divided share of the cost of performing the additional testing of individuals suspected or diagnosed with SARS-CoV-2 and the RT-PCR retesting of previously positive individuals. - The provider will pay a divided share of the costs of the administrative, analytical work and open access publishing. - All funds are collected at a trust account administrated by Hvidovre Hospital, Region Hovedstaden and cost for regional testing is covered from this account. Any excess funds will be divided between the providers when the study is published. The planned RT-PCR testing for SARS-CoV-2 of individuals with suspected COVID-19 infection is conducted as planned by the Danish health-care system
Roles and responsibilities	The study will be open as a national project welcoming all Departments of Clinical Microbiology (DCM) in Denmark, all test centers and all medical companies that wishes to promote antigen tests in Denmark. The steering group will: write and publish the protocol, assess which tests that have the documented quality to be included in the study, oversee the execution of the study, data analysis and drafting of manuscript. The steering group consists of Uffe Vest Schneider, consultant, MD, PhD, DCM Hvidovre Hospital, Jan Gorm Lisby, Chief consultant, MD, PhD, DCM Hvidovre Hospital, Anders Koch, professor, MD, MPH, PhD, Department of Infectious Diseases Rigshospitalet, Nikolai Kirkby, laboratory head, MSc, PhD, DCM Rigshospitalet, and Jenny Dahl Knudsen, head of department, MD, Dr.M.Sci., DCM Rigshospitalet. UVS will draft the protocol with input from the other authors. The steering group will establish a writing group consisting of individuals actively contributing to the study. Providers participating the study will be entered into the protocol and be known to the public. The providers will have access to view data and manuscript 10 days prior to publishing but will not have any influence on the preparation or publication of the manuscript.

	Tests and providers participating in the study: Providers are still being included, but the following vendors are confirmed by April 22th, 2021. • Acro Biotech COVID-19 Ag test, Acro Biotech Inc., 9500 7th Street, Unit M, Rancho Cucamonga, CA 91730, USA • AllTest Covid-19 Ag rapid test, Aidian, Ørestads Boulevard 73, 2300 Copenhagen S, Denmark • Gensure COVID-19 antigen rapid test, Aidian, Ørestads Boulevard 73, 2300 Copenhagen S, Denmark • AUH hjemmetest, Aarhus Universitetshospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N • BD Veritor, Becton Dickinson Denmark A/S, Firskovvej 25B, 2800 Kgs. Lyngby, Denmark • DNA Diagnostic COVID-19 Ag test, DNA Diagnostic A/S, Voldbjergvej 14, 8240 Risskov • Wantai SARS-CoV-2 Ag Rapid Test, Nordic BioSite, Landgreven 3, st.th, 1301 Copnehagen, Denmark • SD Biosensor Standard Q Covid-19 Ag test, Copenhagen Contractors, Sankt Annae Plads 11, 1250 Copenhagen K, Denmark • CoronaCheck, Exhalation Technology Ltd, 42 Newmarket Road, Cambridge, CB5 8EP, UK • Flowflex Acon SARS-CoV-2 Antigen Rapid Test, Acon Laboratories Inc., 10125 Mesa Rim Road, San Diego, CA 92121, USA • COVID-19 Antigen Detection Kit (Colloidal Gold), In-Vitro A/S, Kratbjerg 336, 3480 Fredensborg, Denmark • LumiraDx SARS-CoV-2 Ag test, LumiraDx Limited, 3 More London Riverside, London, United Kingdom • Wholepower COVID-19 Antigen test, Medicotrust A/S, Amaliegade 43, 1.sal, 1256 København K, Denmark • Biosynex Covid-19 Ag/Ag+ BBS (SW400006/SW400010), Medkoncept, Mosehøjvej 7A, 2920 Charlottenlund, Denmark • Noviral COVID-19 rapid cassette antigen test, Noviral International AB, Västmannagatan 3, 11124 Stockholm, Sweden • Mö-screen corona Ag test, Qiagen Denmark, Fruebjergvej 3, 2100 København Ø, Denmark • Egoo.health, Qlife Aps, Fruebjergvej 3 (Symbion), 2100 Copenhagen, Denmark • SARS-CoV-2 Rapid Antigen test, Roche, Industriholmen 59, 2650 Hvidovre, Denmark • Elecsys SARS-CoV-2 Ag test, Roche, Industriholmen 59, 2650 Hvidovre, Denmark • Clinitest rapid COVID-19 Ag test, Siemens Healthineers, Siemens Healthcare A/S, Borupvang 9, 2750 Ballerup, Denmark • CoV2Ag Chemiluminescent immunoassay test kit for COVID-19, Siemens Heathineers, Siemens Healthcare A/S, Borupvang 9, 2750 Ballerup, Denmark • VivaDiag Pro Sars-CoV-2 Ag test, Simoco Diagnostics Aps, Tvedsagervej 30, 3400 Hillerød, Denmark

	• CTK Biotech - Onsite COVID-19 Ag rapid test, SSI Diagnostica, Herredsvejen 2, 3400 Hillerød, Denmark • Fujirebio, Espline SARS-CoV-2 & Quidel Sofia SARS Ag FIA test, Triolab A/S, Vallensbækvej 35, 2605 Brøndby, Denmark • Quidel Sofia SARS Ag FIA test, Triolab A/S, Vallensbækvej 35, 2605 Brøndby, Denmark • Wondfo 2019-nCoV antigen test, Unigroup ApS, Diplomvej 373, 2800 Kgs. Lyngby, Denmark • API Pharma, Vingmed Vicare A/S, Birkerød Kongevej 150B, 3460 Birkerød, Denmark • Szybio SARS-CoV-2 antigen assay kit, Wuhan Life Origin Biotech Joint Stock Co., Ltd, Wuhan Hi-tech Medical Devices Park, Building B11,#818 Gaoxin Road, Donghu Hi-Tech Development Area, Wuhan, Hubei Province 430206, P.R.China DCMs and test centers participating in the study: • DCM Aalborg Universitetshospital, Hobrovej 18-22, 9000 Aalborg • DCM Amager-Hvidovre Hospital #445, Kettegaard Allé 30, 2650 Hvidovre including Department of Clinical Chemistry, Bispebjerg Hospital. • DCM Herlev-Gentofte Hospital, Borgmester Ib Juuls Vej 52, 5. etage, 2730 Herlev • DCM Odense University Hospital, J.B. Winsløws Vej 21, 2. Sal, 5000 Odense C • DCM Region Midtjylland, Aarhus Universitetshospital, Palle Juul-Jensens Boulevard 99 Skejby, 8200 Aarhus N • DCM Rigshospitalet, Henrik Harpestrengsvej 4A, 2100 København Ø • DCM Slagelse Hospital, Ingemansvej 46, stuen, 4200 Slagelse • DCM Sydvestjysk Sygehus, Finsensgade 35, 6700 Esbjerg • DCM Sygehus Lillebælt, Syddansk Universitetshospital, Sygehusvej 24, Beriderbakken 4, 7100 Vejle • Testcenter Danmark, Statens Serum Institut, Bygning 392, Artillerivej 5, 2300 København Ø. • Virus & Microbiological Special Diagnostics, Bygning 87, Artillerivej 5, 2300 København Ø.

Introduction

Background and rationale	Multiple antigen tests targeting SARS-CoV-2 are being marketed commercially, accompanied by reporting of accuracy by the vendor. To evaluate the real-life accuracy of such tests, independent user-initiated studies are needed. Antigen tests are interesting from a public health perspective, as they are rapid (approximately 15-20 minutes) compared to centralized amplification based testing e.g. RT-PCR, but previous antigen tests e.g. for Influenza and Respiratory Syncytial Virus have shown unacceptable low sensitivity when compared to nucleic acid based testing (NAT, e.g. RT-PCR) (Chartrand 2012, Bruning 2017, Merckx 2017). CE marked antigen tests are mostly validated for use with nasopharyngeal swabs. Unfortunately, nasopharyngeal swabbing is normally avoided in adults due to the discomfort for the patient leading to risk of non-compliance and are widely substituted by oropharyngeal swabbing. In the present protocol oropharyngeal

	swabs or less invasive testing e.g. nasal swabs will be used to limit the discomfort for the participating individuals and to reflect the real-life testing approach in adults. An alternative test strategy with saliva swabs with RT-PCR testing will be included as part of the study.
Objectives	To evaluate the analytical and clinical sensitivity and specificity of antigen tests performed as oropharyngeal swabs, outer nasal swabs, saliva swabs, self-test or breath tests for SARS-CoV-2 towards standard RT-PCR testing
Trial design	Prospective accuracy observational study combined with retrospective analytical sensitivity and specificity study.

Methods: Participants, interventions, and outcomes

Study setting	Test centers screening out-patients and hospital staff for SARS-CoV-2 and hospitalized patients positive for SARS-CoV-2. Test centers are invited to participate nationwide. All RT-PCR tests are conducted at the DTU test center and all retrospectively testing of samples by antigen tests is conducted in collaboration between DCM Hvidovre, Rigshospitalet and SSI.
Eligibility criteria	Inclusion criteria. Age 18 years or over, has the capacity to give informed, written consent and be able to cooperate to the additional testing. Exclusion criteria. Individuals not fulfilling the inclusion criteria or declining additional pharyngeal swabbing or saliva swabs.
Interventions	200 consecutive individuals are tested for SARS-CoV-2 by RT-PCR and antigen test. When RT-PCR results are available, additional individuals who has tested positive for SARS-CoV-2 (within 24 hours of positive test result to contact) are invited to participate in the study. SARS-CoV-2 positive individuals are retested until 200 RT-PCR positive individuals and 200 RT-PCR negative individuals have been included for each antigen test. Testing is done with three oropharyngeal swabs at a time in a tree point swab procedure. The three swabs are held together and rotated at both tonsils and at the back of the oro-pharynx. Additional nasal swabs, saliva swabs or breath test will be collected for additional antigen and RT-PCR testing. The consecutive 200 individuals are tested locally by RT-PCR and the residual volume in NEST-buffer is sent to the DTU test center to ensure that all individuals are tested in the same RT-PCR setup. Saliva swabs are when relevant processes according to the manufactures procedure locally and the samples are sent to the DTU test center for RT-PCR. Additional oropharyngeal swabs, nasal swabs, saliva collection, self-test and breath tests are used for antigen testing according to the manufacturer's instructions locally at the test center. Positive patients that are retested, are tested at home / bed-side by an range of antigen tests and one oropharyngeal swab is sent to DTU test center for RT-PCR together with samples from other anatomical test locations when relevant. For self-test tested individuals will send a picture and their interpretation of the test to the test-coordinator. The DTU test center will test for two targets in the Nucleocapsid protein gene (N-gene) of SARS-CoV-2 and for the RNase P gene (RP-gene) from humans to as process control and confirmation of the presence of human DNA in the sample. Excess material from negative patients will be discharged after testing. Excess material from previous positive patients will be discharged. Sample material from

	prospective positive individuals will be treated according to normal practice at the local DCM. For the retrospective study arm, stored excess material from 200 previously positive and 100 negative SARS-CoV-2 samples stored at -80 °C is defrosted on ice and checked by RT-PCR to ensure the Cq value. Selected samples are defrosted again on ice and aliquoted into 250 µL volume and stored at -80 °C until use. For each test one aliquot of each sample is defrosted and 200 µL sample in UTM is tested according to the manufacturer's instruction by laboratory trained personnel in collaboration between DCM Hvidovre, RH and SSI. One aliquot is used for each test and excess material is discharged. One aliquot is retested by RT-PCR after thawing to verify the Cq of the sample.
Outcomes	Primary outcome: Performance of each antigen in relation to the RT-PCR test result. Secondary outcomes: Performance of each antigen test in relation to the cycle of quantification Cq by RT-PCR with and without normalization towards human DNA. The fraction of consecutive samples negative for human DNA by RT-PCR. Agreement between oropharyngeal swabs and tests from other anatomical test locations by RT-PCR. Analytical sensitivity and specificity of each antigen test on retrospectively collected SARS-CoV-2 positive and negative samples. SARS-CoV-2 positive samples are stratified into subgroups according to Cq by RT-PCR.
Participant timeline	Either included when referred to SARS-CoV-2 testing and agrees to participate in the testing or being invited as in-patient, by phone or electronically and agrees to retesting after a positive SARS-CoV-2 RT-PCR test. Invited in- or out-patients positive for SARS-CoV-2 are retested with their consent again as soon as possible after first positive RT-PCR test by an outpatient screening team, who visits and samples each patient at home or for in-patients by trained hospital personnel.
Sample size	Prospective study arm. 200 SARS-CoV-2 RT-PCR positive and 200 SARS-CoV-2 RT-PCR negative patients included for each antigen test. Retrospective study arm. 200 SARS-CoV-2 RT-PCR positive and 100 SARS-CoV-2 RT-PCR negative samples retested for each antigen test.
Recruitment	Individuals with suspected SARS-CoV-2 infection referred to a test center are asked for participation in the study and given the information for participants. Those agreeing for participation in the study sign a consent form. Individuals who test positive for SARS-CoV-2 are invited to participate in the study by either 1) personal contact with hospital personnel for in-patients, 2) by phone or 3) by electronic invitation, which is sent together with the information for participants as a mail in e-boks. The mail is sent to the individual by the laboratory or test-center overseeing the local testing as an invitation for participation. Individuals are asked to contact the local laboratory either by phone or mail, depending on the local setup and to provide a phone number to provide further information and planning of additional testing if relevant. When retested at the hospital or by the out-patient testing team a signed consent form for participation will be collected.

Methods: Data collection, management, and analysis

Data collection methods	RT-PCR result is reported with cycle of quantification Cq for positive tests together with the Cq for human DNA in the sample. Antigen tests are reported according to the manufactor's instruction and divided into positive, negative, inconclusive or invalid tests. Inconclusive and invalid tests

	are handled according to the manufactor's instruction. Each antigen test is photographed to document the result. In the retrospective study arm all antigen tests are tested with stored excess material with seeking 50 samples with Cq 20 to 25, 50 samples with Cq 25 to 30, 50 samples with Cq 30-35 and 50 samples with Cq >35. The 100 negative samples are pooled negative samples that are retested by RT-PCR again prior to use.
Data management	Written consent for participation and collection of data in the study will be collected at the test center and each consent is marked with the local sample identifier number. For consecutive samples each antigen test and the sample for RT-PCR at the DTU test center will be marked with the local sample identifier number and data will be paired according to this sample identification number. Individuals positive for SARS-CoV-2 will be invited in person, by phone or electronically for additional testing and individuals who agrees to additional testing, except in-patients will be contacted using their self-reported contact information's. The number of individuals contacted and rejecting participation will be registered, without additional information. At each retest a new sample identifier number will be pair with the swab(s) for RT-PCR testing at the DTU test center and the antigen tests conducted bedside by trained hospital personnel or outpatient team. Data including photo documentation of antigen test results will be send to the steering group and stored in a secure regional file archive.
Statistical methods	Prospective arm: Performance of each antigen test will be reported as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) compared to oropharyngeal swabs evaluated by RT-PCR. Clinical performance of antigen tests will be further evaluated in respect to strong (Cq <25), intermediate (Cq 25-35) and weak (Cq >35) RT-PCR tests and in respect to samples with low SARS-CoV-2 RNA to human DNA ratio, equal SARS-CoV-2 RNA to human DNA ratio and high SARS-CoV-2 RNA to human DNA ratio. Among consecutive collected prospective samples the fraction of samples negative for human DNA by RT-PCR will be reported. Retrospective study arm: Determination of analytical sensitivity, specificity stratified into four groups by PCR (Cq 20-25, Cq 25-30, Cq 30-35 and Cq >35). Comparison of sensitivity and specificity between antigen test will be done using McNemar chi-squared test. Comparison of PPV and NPPV between antigen test will be done by bootstrap test. 95 % Confidence intervals for sensitivity, specificity, positive predictive value and negative predictive value are calculated as bootstrap confidence intervals.

Ethics and dissemination

Research ethics approval	The regional ethics committee in Region Hovedstaden will be contacted for approval of the involvement of individuals, by direct contact, phone invitation and electronical invitations of SARS-CoV-2 positive patients into the study, bed-side testing by trained hospital personnel or the outpatient team and the intended information for participants in the study. The accuracy of antigen tests needs to be clarified to make clear whether antigen test or alternative test strategies towards SARS-CoV-2 may be used for rapid diagnostics of COVID-19 in the health care system.
Protocol amendments	Protocol modification will be registered, and protocol updates will be shared with the trial registry, antigen test providers and the writing group by the steering group

Consent or assent	Will be collected by staff members from the test centers, hospital personnel and the outpatient testing team
Confidentiality	Written consent for individuals participating in the study will be marked with sample identifier number and will be stored in a secure electronic data structure until a year after publication of results. The hard copy of the consent form will be destroyed after electronic storage. Consent from any individual initially agreeing to the study, but afterwards not meeting the inclusion criteria or retracting consent will be destroyed at the local test center at the latest after inclusion of all study participants at the local test center and only information about the number of individuals retracting consent will be stored.
Declaration of interests	None of the members of the steering committee have any financial or other competing interests in any companies providing antigen test to the study. Members of the writing group will be asked to declare any financial or other competing interests before entering the writing group.
Access to data	The steering group will have access to all data and can share the data as sequential data without local sample identifier numbers with the writing group, statistical experts and participating antigen test providers when data have been collected and analyzed for all participating tests.
Dissemination policy	Authorship will follow the ICMJE recommendations for authorship. The writing group will draft and qualify the manuscript but may include professional English editing to ensure a more fluent and readable manuscript. The results will be published in a peer-reviewed journal as an open access article to ensure full access to the results for any person interested in the study.

Appendices

Informed consent materials	Attachment 1. Cost estimate. Attachment 2. Written consent for participation in the study (in Danish) Attachment 3. Information for individuals participating in the study (in Danish) Attachment 4. Protocol resume