Evaluation of the accuracy of saliva rapid antigen self-testing for SARS-CoV-2 infection

PROTOCOL

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SUMMARY

Rationale: The Dutch public SARS-CoV-2 testing programme considers the introduction of self-collected saliva antigen-detecting rapid diagnostic self-tests (Ag-RDT) for the detection of SARS-CoV-2 infection. If sufficiently accurate, as compared to the prevailing reference test (reverse transcriptase polymerase chain reaction; RT-PCR) and to the prevailing self-collected nasal swab Ag-RDT, the self-collected saliva Ag-RDT can be introduced as preferred self-test since it is less invasive and easier to use than the self-collected nasal swab. This study is very timely since almost all countries are facing the re-opening of societies and public life, where self-testing undoubtedly becomes an integral part of our lives. However, before wide scale implementation of self-collected saliva SARS-CoV-2 Ag-RDT in asymptomatic or symptomatic individuals, quantification of their accuracy (notably their sensitivity and negative predictive value) using the RT-PCR as reference, and with a head-to-head comparison versus the prevailing self-collected nasal swab Ag-RDT, is warranted. Also, evidence of the diagnostic accuracy of the self-collected saliva Ag-RDT across symptom presence, vaccination status, and if possible virus variants Is urgently needed.

Objective: To quantify the diagnostic accuracy of three self-collected saliva Ag-RDT with RT-PCR as the reference standard, and a head-to-head comparison with the prevailing self-collected nasal swab Ag-RDT in The Netherlands (Roche SD-Biosensor; SD-B), in pre-/asymptomatic and symptomatic tested individuals.

Study design: Prospective cross-sectional diagnostic test accuracy study. Each individual scheduled for a routine SARS-CoV-2 RT-PCR test at one of the participating Dutch public health service test sites who provides informed consent, will subsequently receive two Ag-RDT self-tests to apply at home (within a predefined time range): a self-collected saliva Ag-RDT (which differs per participating site) and the Roche SD-B self-collected nasal swab Ag-RDT (since SD-B is currently the prevailing nasal self-test in The Netherlands and has a specific manufacturers' instruction for self-use), both according to the manufacturers' instructions. Participants will also be asked to complete a short online baseline questionnaire, after the two self tests at home have been performed. The RT-PCR routine test results will be communicated to the participants, and is used for any contact tracing in case of a positive result.

Viral culturing will be performed on the RT-PCR specimen and whole genome sequencing of the SARS-CoV-2 virus in all discordant cases (between negative self-collected saliva Ag-RDT and RT-PCR positive cases and between negative self-collected nasal swab Ag-RDT and RT-PCR positive cases).

The reading of both self-tests will be done both by a direct visual interpretation by the study participant and by a dedicated App in combination with a colour reference card provided as part of the self-testing kits.

Study population: Individuals aged 16 years and older presenting for routine SARS-CoV-2 RT-PCR testing at one of the three participating Dutch public health service test sites (West-

Brabant, Rotterdam city and TBD) regardless of test indication, symptomatology, and COVID-19 vaccination status at test request.

Main study parameters/endpoints: The diagnostic accuracy (sensitivity, specificity, positive and negative predictive values) of the three self-collected saliva Ag-RDT as well as the self-collected nasal swab Ag-RDT will be assessed (independently of the result of the self-tests) using RT-PCR as the reference standard. As reference test result we will use both the standard provided RT-PCR test result as well as the RT-PCR test result stratified for the viral load cut-off above which 95% of RT-PCR positives have a positive culture as a proxy of infectiousness). In secondary analyses, the test accuracy of the self-collected saliva Ag-RDT will be stratified according (but not limited) to age groups, gender, having had a positive SARS-CoV-2 test previously, having been vaccinated (and with which COVID-19 vaccine), indication for testing, the presence of symptoms at testing, and SARS-CoV-2 variant. Finally, we will assess the accuracy of the saliva and nasal self-test when read by the study participant themselves versus the reading (blinded for the personal reading) by the app.

Sample Size: Previous Ag-RDTs performance studies showed sensitivities around 85% [5] and when used as a self-test around 80% [https://doi.org/10.1101/2021.02.21.21252153]. We therefore base our sample size calculation on an expected sensitivity of 80% for the self-collected saliva Ag-RDT, with a margin of error of 7%, type I error of 5% and power of 90%. Hence, we aim for approximately 145 positive RT-PCR tests, per self-collected saliva Ag-RDT brand. Based on similar studies on Ag-RDT we expect the number of individuals who will refuse, to be negligible. In the current situation (May 2021), we anticipate a SARS-CoV-2 prevalence (based on RT-PCR) in our target population of around 10% in the tested populations, but we will closely monitor the RT-PCR test positivity rate in our study population over time and prolong recruitment if needed. Hence, we expect to need 4 weeks for patient recruitment.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: There is no direct personal benefit in participation. The participant will undergo the standard swabs for RT-PCR testing, followed by self-collection of saliva for the saliva Ag-RDT and self-collected nasal swab for the nasal SD-B Ag-RDT. The additional burden of the self-tests is negligible; they are minimally invasive and pose no risk to the health of the participant. Participant data will be collected and managed using privacy proof distant electronic data entry tools (Castor).

Generalisability: Currently, self-tests are used in pre- or asymptomatic individuals (e.g., to determine whether one is tested negative to be able to attend public events, restaurants, etc.). Ideally, our study population would therefore be a random sample from the general population, who receives both self-tests and subsequently (within a short time range) independently of the results of the self-tests, the RT-PCR test performed by trained personnel. This design is however unfeasible for various reasons. First, the prevalence of

SARS-CoV-2 in the general population is currently less than 0.7%, which in our case would require a very large sample size and therefore result in a undesirable long recruitment period. Second, the number of missing RT-PCR (reference test) results will be very large since individuals are much less likely to undergo the more invasive reference standard test after obtaining the self-test results; such a large number of missing outcome data will severely affect the validity of the study. Third, logistically it will be much harder to recruit study participants from the general population and deliver both self-tests with instructions to their homes, as compared to recruitment at the dedicated test sites. Hence, we explicitly chose for a design where study participants are selected at the testing sites. To mimic as much as possible the target population of self-tests every individual representing at the test sites will be eligible. Our study will therefore include individuals with symptoms or a-/presymptomatic close contacts informed via the test-and-trace program ('BCO' in Dutch), the CoronaMelder app, or by an index case. Asymptomatic non-close-contact individuals will, for the reasons mentioned above, explicitly not be part of this study. The self-collected saliva Ag-RDT accuracy parameters estimated from our study population recruited at the test sites will be extrapolated to their expected accuracy in the general population. Such extrapolation is not uncommon to do. We will apply so-called prevalence adjustment techniques, to adjust the test accuracy parameters estimated from the data (with a current prevalence of about 10% in our tested population) to the current prevalence in the general population (of about 0.6 to 0.7%). Finally, we will assess the reliability of an App that reads the results of the test automatically. This could result in a more objective way of reading the results and may also be connected to a platform for digital exchange of test results. This is important as the Dutch government and minister of Health aims to explore the possibility for self-tests to be used as a permit for admission to festivals and for travel.