STUDY PROTOCOL

Evaluation of SARS-CoV-2 Rapid Antigen Test: increasing testing capacity in screening of SARS-CoV-2 (SARA)

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Independent expert (s)	5.1.2e **T:** 5.1.2e
Enrolling sites	***GGD test lanes Amsterdam and Rotterdam Rijnmond***

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

COVID-19	CORonavirus Infectious Disease
RT-PCR	Reverse transcriptase Polymerase Chain Reaction
RAT	Rapid Antigen Test
SARS-CoV-2	SARS Coronavirus 2
WHO	World Health Organization
GGD	Municipal Health Center
UMCU	University Medical Center Utrecht
EMC	Erasmus Medical Center
RIVM	National Institute for Public Health and the Environment
LAMP	loop-mediated isothermal amplification

SUMMARY

Rationale: Good and rapid diagnostics are essential for the treatment and control of COVID-19. The current testing regimes relies on active case finding of COVID-19 infection using real-time reverse transcriptase PCR (RT-PCR). RT-PCR is highly sensitive and specific with results obtained within 24 hours. However, control of the pandemic has required countries to drastically scale up their testing capacities in the first wave of the COVID-19 pandemic. Nonetheless the capacity is not enough as the current testing regimes takes at least 24-48 hours from sample to result and this time increases in high prevalent regions.
As such there is an increasing need and demand for rapid test currently being marketed to be used. Reliable rapid diagnostic tests could reduce the pressure on laboratories, GGD and expand testing capacities.

The aim of this protocol is to perform clinical evaluation of several promising Rapid Antigen tests (RATs) against the standard molecular diagnostic assay RT-PCR directly in the field within the GGD COVID19 test lanes.
Objective: Primary objective is to determine the diagnostic performance on sensitivity and specificity of the RAT compared to RT-PCR and usability at GGD test lanes. Secondary objectives are to determine the analytical and diagnostic performance of sensitivity and specificity compared to RT-PCR stratified by viral load/Ct values, disease stage, asymptomatic and severity of symptoms, sample type, prevalence of SARS-CoV-2 in population tested, age and sex.
Study design: Prospective clinical evaluation study and prospective and retrospective lab based evaluation.
Study population: We aim to include 3000-4000 cases visiting GGD test lanes for COVID-19 testing by RT-PCR within the study period and who are willing to participate in the study through informed consent.
The sample size may be adjusted based on the results that are obtained.
Main study parameters/endpoints: Diagnostics performance of assays on sensitivity and specificity. RAT that meet the minimum performance criteria of >80% sensitivity and >97% specificity will be selected for advised specific screening purposes such as outbreak management testing.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participation in this study poses a negligible risk and the burden is considered minimal. Nasal/throat swabs are commonly used methods for collecting test samples for respiratory viral infection. Swabbing may be mildly uncomfortable as it may cause momentary gagging. Discomfort and risk will be minimized by having experienced personnel take the swabs. There is no direct benefit to the subjects.

1. INTRODUCTION AND RATIONALE

Good and rapid diagnostics are essential for the treatment and control of COVID-19. The current testing regimes relies on active case finding of COVID-19 infection using real-time reverse transcriptase PCR (RT-PCR). RT-PCR is highly sensitive and specific with results obtained within 24 hours.

The measures to control the COVID-19 pandemic rely heavily on fast and accurate testing of (suspected) COVID-19 cases and their contacts. SARS-CoV-2 testing is intended to identify current infection in individuals and is generally performed when a person has signs or symptoms consistent with COVID-19, or when a (asymptomatic) person has been in contact with a SARS-CoV-2 positive case or identified by a SARS-CoV-2 positive case through contact tracing.

However, control of the pandemic has required countries to drastically scale up their testing capacities in the first wave of the COVID-19 pandemic. Nonetheless the capacity is not enough as the current testing regimes takes at least 24-48 hours from sample to result and this time increases in high prevalent regions.

As such there is an increasing need and demand for rapid test currently being marketed to be used. Reliable rapid diagnostic tests could reduce the pressure on laboratories, GGD and expand testing capacities. However data is limited on the performance of these assays in GGD test lanes.

Rapid testing will allow for more rapid and informed quarantine guidelines, in particular for priority professions. These tests will also enable alleviation of containment measures enabling (partial) restoration of high societal and economic impact the pandemic has had.

The aim of this protocol is to perform clinical evaluation of several promising Rapid Antigen tests (RATs) against the standard molecular diagnostic assay RT-PCR directly in the field within the GGD COVID19 test lanes

Principle Antigen Rapid Test (RAT)

Rapid Antigen Test are designed to quickly test SARS-CoV-2 suspected cases in 10-15/30 minutes.

Rapid Antigen Rapid Tests are lateral flow assays (LFA) (figure 1) and are based on the detection of SARS-CoV-2 antigens **(typically part of N or S protein**). Antigens are structural molecules present on the outside of the virus and that can react to host immune components. Antigen can be specific for a virus family (*Coronaviridae*), subgenus (Sarbecovirus), lineage (B), type (SARS-CoV-2). As such the Antigen used in Antigen Rapid Test should be virus type specific as a-specific binding to on the circulating coronaviruses, may lead to false positive results.

RATs are easy to use and can be performed by Colloidal Gold analysis (no device required) and two will be based on automated analysis (device required). The advantage of automated analysis is the objective read out.

The test is designed to provide a **qualitative** yes (two lines, test control and virus) or no (one line, test control) answer. The intensity of the bands and visual detection of two lines is dependent on the concentration of the antigen in the patient material. The performance of

these tests is dependent on the sensitivity/specificity of these tests as well as the prevalence of SARS-Cov-2 in the target population. Overall, Antigen Rapid tests are less sensitive compared to molecular assays (https://www.finddx.org/covid-19/dx-data/). As such the usability of these test should be evaluated with regards to the aim and situation at hand (e.g. rapidly identifying cases in a suspected outbreak) and whether additional confirmation is needed by RT-PCR (https://www.who.int/publications/i/item/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19-scientific-brief).

2. OBJECTIVES

Primary Objective: Determine the diagnostic performance (sensitivity and specificity) and usability of RAT against standard PCR in the GGD test lane. The data will be stratified to viral load/ct value standardized among laboratories.

Secondary Objective(s): Asses the diagnostic performance according to:

- Disease stage/ days after onset of symptoms
- Asymptomatic or symptomatics
- severity of symptoms
- sample type
- Prevalence/cohort
- Age and sex
- turnaround time/sample to result

3. STUDY DESIGN

We propose a prospective evaluation study within GGD test lanes. GGD test lanes currently coordinate COVID-19 testing of (mild) symptomatic cases in the Netherlands.

4. STUDY POPULATION

4.1. Population

- <18 years
- Not agreed to participate (informed consent)

4.4. Sample size calculation

We aim to include 3000-4000 cases visiting the GGD testlanes during the study period

5. TREATMENT OF SUBJECTS

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1. Study parameters/endpoints

Diagnostics performance of assays on sensitivity and specificity of RAT compared to RT-PCR performance. RAT that meet the minimum performance criteria of >80% sensitivity and >97% specificity will be selected for advised specific screening purposes such as outbreak management testing.

8.2. Study procedures

Enrolment:

- According to the testing protocol of the GGD test lanes, a person with a test indication makes an appointment to be tested at a test lane.
- All persons visiting the test lane will be given asked to participate within the study and given
  - A full participant information letter with an informed consent form to be signed.
  - A clinical enquiry questionnaire is given to be filled in to collect symptom information.

Sampling and testing:

- One nasopharyngeal swab and one oropharyngeal swabs for RT-PCR and one nasal swab or NSP for RAT are performed by trained GGD personnel and sent to the regional laboratory for RT-PCR and RAT.

Reporting

- Laboratories electronically report RT-PCR test results within (preferably) 24 hours to the GGD trough a digital communication system, the tested individual will thereafter be informed by a GGD employee of the RT-PCR results.
- Results for RAT and second PCR are not reported to the person as the results are under evaluation and will not provide additional information to the person.
- RAT and RT-PCR results will be reported to RIVM via a secure portal/database.

8.3. Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.3.1. Specific criteria for withdrawal (if applicable)

Not applicable

8.4. Replacement of individual subjects after withdrawal

Not applicable

8.5. Follow-up of subjects withdrawn from treatment

Not applicable

8.6. Premature termination of the study

Not applicable

9. SAFETY REPORTING

Nasal/throat swabs are currently performed in routine diagnosis. The study involves an additional nasal/throat swab and nasopharyngeal swab. Due to the negligible risk of this procedure, no adverse events will be reported.

10. STATISTICAL ANALYSIS

The data includes categorical values and will be presented as quantitative data using standard statics for categorical data.

11. ETHICAL CONSIDERATIONS

11.1. Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki, amended at the 64th General Assembly (Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and the Code Goed Gebruik (2011).

11.2. Recruitment and consent

Information signs will be placed at the participating test lanes and the study will be announced through the RIVM and GGD website as well as through social media and news channels.

Individuals presenting at the test lanes will be informed by a full participant information letter with informed consent will be handed over to be read and signed on sight.

11.3. Objection by minors or incapacitated subjects (if applicable)

Not applicable

11.4. Benefits and risks assessment, group relatedness

Participation in this study poses a negligible risk and the burden is considered minimal.
Nasal/throat and nasopharyngeal swabs are commonly used methods for collecting test samples for respiratory viral infection. Swabbing may be mildly uncomfortable as it may cause momentary gagging.
Discomfort and risk will be minimized by having experienced personnel take the swabs.
There is no direct benefit to subjects.

11.5. Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6. Incentives (if applicable)

Subjects will not be compensated for participating in this study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1. Handling and storage of data and documents

Data will be handled confidentially in compliance with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet AVG, UAVG).
RAT and RT-PCR results will be reported in pseudonymized fomat to RIVM via a secure portal/database .
Only aggregated data will be reported to collaborating and fundingbodies. All files will be password protected. Data can only be used as described here. Data will be stored for 20 years after the completion of this study according to WGBO (wet geneeskundige behandelingsovereenkomsten) article 4.6/ 8.1.7.

12.2. Monitoring and Quality Assurance

Participating laboratories are accredited to conduct the SARS-Cov-2 PCR assay.

12.3. Amendments

All amendments will be notified to the participating parties and sponsors and to the acting METC of the participating parties that the amendment is still in line with nWMO.

12.4. Public disclosure and publication policy

The data from the field evaluation will be compared with data from collaborating institutes. A data sharing agreement will be signed between all parties involved. The investigators will report the results of the study during and at the end of the study to the sponsor. The study will be registered in the Netherlands Trial Register before the first subject is recruited.

13. STRUCTURED RISK ANALYSIS

Not applicable