SARSLIVA: utility of saliva in COVID-19 diagnosis - a household study

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NL74555.029.20 SARSLIVA

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
DSMB	Data Safety Monitoring Board
EudraCT	European drug regulatory affairs Clinical Trials
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
(S)AE	(Serious) Adverse Event
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale:

In mitigating the SARS-CoV-2 pandemic, governments have to weigh the public health benefit of interventions such as closure of schools, restaurants and theatres against the significant societal and economic disruption they impose. After opening up schools and public life, and restarting the economy, close monitoring of spread of the virus is a crucial tool for success of tracing the virus and SARS-CoV-2 spreading in the population. Recently, tracking the virus in saliva by molecular diagnostics was shown to be at least as sensitive as the most broadly used method of viral detection in nasopharyngeal (NP) swabs (Wyllie et al., 2020). Saliva sampling would be a very attractive way for large scale monitoring of SARS-CoV-2 spreading, mostly because it gives the possibility of self-sampling in the home situation. However, saliva sampling has not yet been validated for asymptomatic and pre-symptomatic SARS-CoV-2 infected individuals. If saliva sampling would be a reliable method for early detection, containment of viral spread would be easier. Saliva has also not yet validated at lower viral density in the track of SARS-CoV-2 infection. This would allow us to investigate whether persons, who we now know may remain symptomatic for a prolonged period of time (weeks to months), continue to shed the virus and may still be infectious. In addition to SARS-CoV-2 detection, saliva may also be a good specimen for detecting emerging mucosal IgA, IgM and IgG antibodies against SARS-CoV-2 (Randad et al., 2020). Also, with molecular diagnostics, other respiratory viral and bacterial pathogens can be detected in saliva (Krone et al., 2015; Rodrigues et al., 2019; Wyllie et al., 2014). Lastly, saliva may be a tool for oral microbiome and mycobiome analysis.

Objectives:

Primary objectives

1. The sensitivity of saliva collection for SARS-CoV-2 tracing, with home self-sampling, storage and transport to the laboratory.
2. The emergence of SARS-CoV-2 in saliva from asymptomatic or pre-symptomatic household contacts, duration of the presence of SARS-CoV-2 in saliva, and the changes in viral load over time in relation to disease severity.
3. The emergence of IgA, IgM and IgG anti-SARS-CoV-2 specific antibodies in saliva and serum.

Secondary objectives

1. Evaluation of tracing other respiratory viruses in saliva.
2. Evaluation of co-infection with respiratory bacterial pathogens in NP, OP swabs and in saliva by conventional (culture-based) and by molecular diagnostics (PCR).

Exploratory objective

1. Evaluation of the upper respiratory microbiome with 16S and mycobiome with 18S/28S/ITS sequencing

Study design:

This will be a prospective cohort study of patients with laboratory-confirmed COVID-19 and their household contacts. All participants will repeatedly self-collect saliva samples at 10 time points. Research nurses will collect additional NP and OP swabs 7 days following COVID-19 diagnosis of the index patient from all participants. Furthermore, we will use questionnaires to track symptoms of COVID-19. Six weeks after the positive test result of the index patient, we will also collect a capillary blood sample by a finger prick.

Study population:

We will invite index patients with their families on the day they are diagnosed to participate in the SARSLIVA study. The index-patient is diagnosed with (laboratory-confirmed) COVID-19 by routine diagnostics using NP/OP swabs in patients with COVID-19 like symptoms by either 1. the Spaarne Gasthuis (after referral), 2. the general practitioner, or 3. the municipal health services Kennemerland.

Inclusion criteria:

- Age index case 0-65 years, and
- Laboratory-confirmed COVID-19 of index case, and
- Household living together with at least 3 individuals, including the index patient, and
- Informed consent of index patient and at least 2 other household members

Exclusion criteria:

- Not able to read a Dutch patient information leaflet, or
- Living more than 50 km from the Spaarne Gasthuis

Study procedures:

Patients who meet the inclusion criteria and who have indicated to be willing to be approached, will be asked by the study staff to participate in the study together with at least 2 of their household members. Index patients and household members undergo the same study procedures. First, a baseline questionnaire on household composition, household characteristics (number of bedrooms, sharing of bed(room)s, number of toilets, presence of washing basins and presence of pets), smoking, medical history, medication use, vaccination

status, education level, profession, recent contact with people outside of the household (with and without respiratory complaints/proven COVID-19) and recent travel will be collected. During the six weeks following the positive test result of the index case, saliva samples will be self-collected and stored in the freezer in the morning of days 1, 3, 5, 7, 10, 14, 21, 28, 35 and 42. From the age of 5 years, saliva self-collection will be done by drooling (3 ml) into 2 tubes. Below that age, drooling is not possible, and 3 Oracol sponges that children keep in the mouth for about 1 minute each, will be used instead. The night before sampling days, a short questionnaire on the presence and severity of symptoms will be collected. Additionally, on day 7, a health care worker will obtain an additional NP and OP swab, which will directly be tested for presence of SARS-CoV-2. On day 42, at the end of the study, all samples will be collected by a research nurse who will also (assist with) collect(ing) a capillary blood sample. Participants will then also be asked to evaluate the study procedures in a final short online questionnaire.

Main study parameters/endpoints:

To address the primary objectives, the following parameters will be evaluated:

- The sensitivity of testing for SARS-CoV-2 in saliva compared to routine diagnostics performed on NP or OP swabs, or a combination of the two, as well as to NP and OP swabs taken on day 7 after the SARS-CoV-2 positive test result of the index patient.
- The emergence of SARS-CoV-2 in saliva from asymptomatic or pre-symptomatic household contacts, duration of the presence of SARS-CoV-2 in saliva, and the changes in viral load over time.
- Clinical symptoms and severity of disease, i.e. the type and severity of symptoms, duration of symptoms, requiring hospitalization for supportive care and the duration of hospitalization, and requiring ICU admission for mechanical ventilation.
- The emergence of SARS-CoV-2 specific IgA, IgM and IgG antibodies in saliva over time, and the concordance between salivary and serum antibodies.

To address the secondary objectives, the following will be evaluated:

- Presence of other respiratory viruses in saliva.
- Presence of respiratory bacterial pathogens in respiratory samples (NP and OP swabs taken at day 7 and in saliva over time) when detected by conventional diagnostic culture or molecular methods.

To address the exploratory objectives, the following will be evaluated:

- The composition of the upper respiratory microbiome by 16S sequencing and metagenomics.

- The composition of the upper respiratory mycobiome by 18S/28S/ITS sequencing and metagenomics.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness

The burden of participating in this study is minimal. Blood collection will take place only once and will be performed by a finger prick, which may cause transient mild discomfort and only rarely infection. NP and OP swabs are also collected only once. NP swabbing gives mild discomfort, may induce sneezing, and may give a nosebleed in rare cases. OP swabbing also gives mild discomfort and may induce a gagging reflex. Saliva collection holds no risk. Saliva sampling will be repeated in total 10 times for SARS-CoV-2 positive index subjects and household members, but represents no burden also when repeated. Participants will also be required to complete a total of 12 questionnaires, which is expected to take 12x5 minutes maximum per person in the household. The 2 home visits are also estimated to take 5 minutes per person in the household. Study staff will drop off required sampling material and pick up saliva samples at participant homes.

There is no clear clinical benefit for the subjects participating in the study. It is important to note that study participants should still follow the advice from the municipal health services regarding testing for SARS-CoV-2. The study physician will also inform participants about the results on SARS-CoV-2 testing from the NP and OP swab obtained on day 7 by phone as soon as results are available. Testing of these swabs will be performed immediately. At the end of the study, the study physician will also inform participants on the results from the saliva samples, which are not analysed directly. Study procedures will also be evaluated by all participants, leaving room for suggestions.

1. INTRODUCTION AND RATIONALE

In mitigating the SARS-CoV-2 pandemic, governments have to weigh the public health benefit of interventions such as closure of schools, restaurants and theatres against the significant societal and economic disruption they impose. After opening up schools and public life, and restarting the economy, close monitoring of spread of the virus is a crucial tool for success of tracing the virus and SARS-CoV-2 spreading in the population. Recently, tracking the virus in saliva by molecular diagnostics was shown to be at least as sensitive as the most broadly used method of viral detection in nasopharyngeal (NP) swabs (Wyllie et al., 2020). Saliva sampling would be a very attractive way for large scale monitoring of SARS-CoV-2 spreading, mostly because it gives the possibility of self-sampling. However, saliva sampling has not yet been validated for asymptomatic and pre-symptomatic SARS-CoV-2 infected individuals. If we can use saliva for early detection, containment of viral spread is made easier. Saliva is also not yet validated at lower viral density in the track of COVID-19 infection. This would allow us to see whether persons, who we now know may remain symptomatic for a prolonged period of time (weeks to months), continue to shed the virus and may still be infectious.

Nasopharyngeal swabs are currently the specimen most often collected for SARS-CoV-2 detection. However, good NP swabbing requires trained health-care professionals wearing protective equipment, since swabbing induces coughing and sneezing (Kinloch et al., 2020). In contrast, collection of saliva is easy by self-sampling, causes no discomfort and this way avoids the necessity of trained health care workers. Saliva collection can be done at home by COVID-19 patients in follow-up, and by members of household of COVID patients or by those who have been in contact with a case and need to be monitored.

In the study by Wyllie *et al.*, saliva was collected by drooling in a sterile cup in the early morning, before tooth brushing and breakfast, and transported to the laboratory within 48 hours (Wyllie et al., 2020). In a direct comparison between 38 paired nasopharyngeal and saliva samples from COVID-19 patients, they found comparable or higher sensitivity and similar or higher SARS-CoV-2 loads in saliva than in nasopharyngeal swabs. Also, 98 asymptomatic healthcare workers were evaluated and SARS-CoV-2 was found in saliva of two individuals who had tested negative using the nasopharyngeal swabs. In an Italian study by Azzi *et al.*, saliva tested positive for all 25 COVID-19 patients, even on the day that upper respiratory swabs converted to negative (Azzi, Carcano, et al., 2020). These findings along similar findings reported by the same group and by others (Azzi, Baj, et al., 2020; Williams et al., 2020; Zhu et al., 2020) suggest that saliva may be a valid sample for assessing the presence as well as the duration of SARS-CoV-2 infections (Wyllie et al., 2020; Zhu et al., 2020).

Based on our own experience and published papers, a larger volume of saliva collected by drooling seems not only the simplest but possibly also the optimal approach in SARS-CoV-2

detection (Azzi, Carcano, et al., 2020; Williams et al., 2020; Wyllie et al., 2020; Zhu et al., 2020). Almost any modification, like using swabs or any other cotton- or dacron-based devices, with potential inhibitors and less volume of saliva, seem to harm rather than help (5.1.2e , one of authors of the "Brief Summary on Using Oral Fluids for CoV"). In children under 5 years of age, a sponge for saliva collection is required since drooling or spitting is too difficult. In the Netherlands, the currently ongoing FXX study by RIVM collects saliva with an ORACOL sponge, along with NP and OP swabs by trained research nurses at home visits. Saliva is immediately put on ice and samples are transported the same day to the RIVM laboratory. It was confirmed that with this protocol, results from saliva on SARS-CoV-2 detection are virtually in the same range as detection in NP and OP swabs (pers. communication 5.1.2e , RIVM).

For large scale monitoring, home self-sampling without need of health care workers is preferred. In this study, we want to assess the sensitivity of self-sampling of saliva in home situations, compared with routine diagnostics by NP and/or OP swabs in COVID-19 patients. Sampling and storage in home settings should also allow for a delay in transport to the lab for two or more days.

Next, we would like to assess whether saliva sampling is suitable for detecting lower viral loads and tracing SARS-CoV-2, not only in asymptomatic or pre-symptomatic persons who nevertheless may be shedding SARS-CoV-2 and spreading the virus, but also in follow-up of COVID-19 patients for several weeks who may continue shedding via respiratory droplets.

In addition to SARS-CoV-2 detection, saliva may also be a good specimen for detecting emerging mucosal IgA and IgG antibodies against SARS-CoV-2.

Also, with molecular diagnostics, other respiratory viral and bacterial pathogens can be detected in saliva. Lastly, saliva may be a tool for oral microbiome and mycobiome analysis.

2. OBJECTIVES

Primary objective

1. The sensitivity of saliva collection for SARS-CoV-2 tracing, with home self-sampling, storage and transport to the laboratory.

2. The emergence of SARS-CoV-2 in saliva from asymptomatic or pre-symptomatic household contacts, duration of the presence of SARS-CoV-2 in saliva, and the changes in viral load over time in relation to disease severity.

3. The emergence of IgA, IgM and IgG anti-SARS-CoV-2 specific antibodies in saliva and serum.

Secondary objective

1. Evaluation of tracing other respiratory viruses in saliva.
2. Evaluation of co-infection with respiratory bacterial pathogens in NP, OP swabs and in saliva by conventional (culture-based) and by molecular diagnostics (PCR).

Exploratory objectives

1. Evaluation of the upper respiratory microbiome with 16S and mycobiome with 18S/28S/ITS sequencing.

3. STUDY DESIGN

Recruitment:
We will recruit patients with COVID-19 up to 65 years old with a household that consists of at least 3 people including the index case. We will recruit at different sites, through the Spaarne Gasthuis in Haarlem, the general practitioners (GP) in the region of Hoofddorp and Haarlem, and the municipal health services Kennemerland. At all sites, recruitment material will be present (leaflets and/or posters). Eligible subjects with laboratory-confirmed COVID-19 will be asked if they are willing to participate when the positive test result is communicated to them as a part of the routine diagnostic procedures. This will be done by staff from the Spaarne Gasthuis or from the municipal health services Kennemerland, or by the potential participant's GP, depending on who requested the test. If they agree, contact details from potential participants will be communicated to the study team by phone or secure e-mail. Next, on the same day if possible, the study team will approach the potential participants, inform them about the study and answer questions, and send them the patient information leaflet with informed consent form via e-mail. This corresponds to day 0 of the study. When they are interested to participate, study staff will drop off material and obtain written informed consent from them and at least 2 of their household members on the next day (day 1, at least 12 hours later). At this time, study staff may again answer questions. Because of COVID-19, study staff will be required to keep a distance and will not immediately sign the form. Instead, staff will take the signed forms and keep them in a sealed bag for 72 hours. After this time, study staff will also sign the form and return a copy to the study participants. For children below the age of 12 years, both parents/caregivers need to give written consent. For children 12-15 years of age, both parents/caregivers and the child need to give written consent.
Sample collection:
Index patients and household members will undergo the same study procedures on the same days. For those 5 years and older, 2 saliva samples will be self-collected by drooling at least 3 milliliters into an empty, sterile tube, and into a similar tube pre-filled with 0.5 ml DEPC water with 50% glycerol. The second tube contains glycerol, which supports survival of viable microorganisms during storage. We will ask for a volume of at least 3 milliliters per tube. Both saliva tubes are placed in a sealable plastic bag and stored at home in the freezer. After transport to the laboratory, samples with 3 ml of raw saliva will be thawed and split into two aliquots: one for PCR analysis of respiratory viruses (2 ml) and one (1 ml) for antibody detection. The second saliva sample with glycerol will be stored frozen at -80°C until analysis.
For children below the age of 5 years, who cannot be asked to spit into a tube, we will use 3 Oracol 'lollypop' sponges that the children hold in their mouth, one at the time, for at least 1

minute, so that the sponge becomes saturated with saliva. For viral PCR and antibody testing, 2 Oracol sponges are required to obtain a sufficient volume. The third Oracol sponge will be placed in a tube pre-filled with 0.5 ml DEPC water with 50% glycerol. The 3 Oracols are then also placed in a sealable plastic bag and stored at home in the freezer. After transport to the laboratory, raw saliva samples will be thawed, centrifuged and split into two aliquots: one for PCR analysis of respiratory viruses and one for antibody detection. The third Oracol on glycerol will be thawed, centrifuged and stored frozen at -80°C until analysis. Saliva samples will be collected in this manner at 10 time points, namely on days 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42 from the positive SARS-CoV-2 test result of the index patient. Participants will be instructed to collect samples in the morning, before tooth brushing, eating drinking, or smoking. In the raw saliva samples, presence and load of SARS-CoV-2, as well as concentrations of SARS-CoV-2 specific IgA, IgM and IgG will be determined. Also, other respiratory viral infections will be determined. In the samples on glycerol, bacterial and fungal presence will be explored.
On day 7 after the positive test result of the index patient, a research nurse will conduct a home visit (wearing appropriate protective gear, i.e. gloves, mask, coat, and glasses), to collect an additional NP and OP swab from all participants. Copan Eswabs will be used, which will immediately be placed in Amies medium. This is to investigate if SARS-CoV-2 is (still) detected in these NP and OP samples when compared with the saliva sample collected at this same day. These samples will be tested for the presence of SARS-CoV-2 by PCR immediately following transport to the laboratory.
On day 42 after the positive test result of the index patient, a capillary blood sample will be collected using a finger prick. Participants can collect the blood sample themselves, but a research nurse can assist when preferred. This blood sample serves to compare the emergence of SARS-CoV-2 specific antibodies in serum and saliva.

Questionnaires and clinical data:
At the start of the study (i.e. day 1 from the positive test result of the patient), index patients and household members will complete an online questionnaire on baseline characteristics. The baseline questionnaire contains questions on household composition, household characteristics (number of bedrooms, sharing of bed(room)s, number of toilets, presence of washing basins and presence of pets), smoking, medical history, medication use, vaccination status, education level, profession, recent contact with people outside of the household (with and without respiratory complaints/proven COVID-19) and recent travel (in- and outside of The Netherlands). Furthermore, on the nights before saliva is collected (i.e. days 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42 from the positive test result from the index patient), they are asked to complete short online questionnaires on the presence and severity of respiratory and other

COVID-19 like symptoms. In these questionnaires, participants are also asked if they visited the general practitioner (and if yes, on which date and the name of the general practitioner) or the hospital (and if yes, on which date and the name of the hospital). Medical records on visits to the general practitioner, hospital visits or admissions for COVID-19 will then be requested when applicable. We will record routine care data such as clinical signs and symptoms, results from viral PCR or culture for other respiratory viruses, results from routine blood tests, results of blood culture and chest X-ray and CT scan if performed, treatment with antibiotics, oxygen or other medications initiated for COVID-19, complications and duration of hospitalization when applicable.
After the study, procedures are evaluated by participants using a short questionnaire, leaving room for suggestions. This serves to improve the methods and procedures to optimize future (self-)sampling at home for applications in medical practice.

4. STUDY POPULATION

a. Population

The study population will consist of patients with laboratory-confirmed COVID-19 based on routine diagnostics of PCR on NP and/or OP swabs, and (at least 2 of) their household members. Index patients will be recruited after a SARS-CoV-2 positive test result, either at the Spaarne Gasthuis, or via the municipal health services Kennemerland, or through the general practitioners.

b. Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age index case 0-65 years, and
- Laboratory-confirmed COVID-19 of index case, and
- Household living together with at least 3 individuals, including the index patient, and
- Informed consent of index patient and at least 2 other household members.

The age criterion is chosen because the study is designed to study among others transmission of SARS-CoV-2 within households where different generations live together. Therefore, we wish to include mainly families with (young) children, who do not commonly live together with individuals above the age of 65. Without an age criterion, for example, nursing home residents with a communal living area would also be eligible, but we are not interested in this age group.

c. Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Not able to read a Dutch patient information leaflet; or
- When a patient lives outside of the service area of the Spaarne Gasthuis, i.e. outside a radius of 50 km.

d. Sample size calculation

Regarding the sensitivity of SARS-CoV-2 testing in saliva samples, and assuming an estimated sensitivity of 90% (To et al., 2020), the 95% confidence interval of this estimated sensitivity can be calculated using the Wilson Score interval method (Brown et al., 2001). With an expected total of 85 SARS-CoV-2 positive participants who will be included in this study (which is feasible with 75 COVID-19 patients and their family members), this 95% confidence interval will range from [0.83 to 0.95] which we consider adequate to reliably

answer our primary research question. Moreover, data will be further enriched with data from other household studies, i.e. the RIVM FFX study and the ZonMW financed CoKids study. Investigators from RIVM participate in all 3 studies. The Spaarne Gasthuis and Streeklab Haarlem participate in CoKids and SARSLIVA studies. The CoKids study is a household study focused on households with young children, and all members are repeatedly screened for presence of SARS-CoV-2 in nose, throat and saliva samples. When a member of the household is infected with SARS-CoV-2, extra samples (nose and throat swabs, saliva and feces) are collected on days 1, 8 and 15. In saliva samples, presence of SARS-CoV-2 as well as the level of anti-SARS-CoV-2 antibodies are determined, which may be compared to our data. The FFX study is also a household study, where participants are approached after a positive SARS-CoV-2 test at the municipal health services. Participants and their household members are sampled, including saliva, which is repeated after 2-3 and 4-6 weeks. Together, these 2 studies span the entire study period of SARSLIVA. They use a slightly different sampling method, with ORACOL sponges for all age groups, but results will still be comparable.

5. TREATMENT OF SUBJECTS

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

a. Study parameters/endpoints

i. Main study parameters/endpoints

To assess the primary objectives, the following will be evaluated:

- The sensitivity of SARS-CoV-2 tracing in saliva compared with NP and OP swabs will be calculated to study if SARS-CoV-2 is detected just as well in saliva as in NP and OP swabs. Additionally, presence of SARS-CoV-2 in NP and OP swabs taken 7 days after SARS-CoV-2 diagnosis of the index case is compared to presence of SARS-CoV-2 in saliva from the same day.
- The emergence of SARS-CoV-2 in saliva from asymptomatic or pre-symptomatic household contacts, duration of the presence of SARS-CoV-2 in saliva, and the changes in viral load over time.
- Clinical symptoms and severity of disease, i.e. the type and severity of symptoms, duration of symptoms, requiring hospitalization for supportive care and the duration of hospitalization, and requiring ICU admission for mechanical ventilation.
- Antibodies against SARS-CoV-2 in saliva (immunoglobulins A, M and G) and serum (immunoglobulins A, M and G) will be assessed. Emergence of salivary antibodies will be traced over time. To study correlation between serum and saliva antibodies, serum antibodies will be measured at a single timepoint (day 42), which will be compared to salivary antibody concentrations at the time. Testing for SARS-CoV-2 specific IgA, IgM and IgG antibodies in saliva will also potentially help identify additional cases negative on molecular diagnostics and may contribute to trace contact with SARS-CoV-2 by household members.

ii. Secondary study parameters/endpoints

To address the secondary objectives, the following will be evaluated:

- Viral co-infections (i.e. other common respiratory viruses) will be assessed in saliva;
- Bacterial co-infections/colonization with respiratory bacterial pathogens *Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Haemophilus influenzae* and *Neisseria meningitidis* will be assessed in NP, OP swabs and saliva using conventional (culture-based) and molecular methods.

iii. Exploratory study paramters/endpoints

To address the exploratory objectives, the following will be evaluated:

- The upper respiratory microbiome will be assessed using 16S ribosomal RNA gene sequencing at the RIVM.
- The upper respiratory salivary mycobiome will be assessed using 18S/28S/ITS sequencing at the RIVM.

b. Randomisation, blinding and treatment allocation

Not applicable

c. Study procedures

Sample collection:
Saliva samples will be obtained by participants themselves at home. All necessary sampling material will be dropped off by study staff taking necessary safety precautions: staying outside, keeping a distance of at least 1.5 meters, and wearing gloves and mask. Saliva is collected using different methods depending on the age of the participant: 1) by spitting into 2 Isohelix Genefix Saliva Collection devices, 1 empty and 1 pre-filled with 0.5 ml DEPC water with 50% glycerol, using funnels to ensure clean collection, for participants from the age of 5 years, or 2) by placing an Oracol sponge in the mouth for approximately one minute, rubbing it against the gums, and placing this in a tube for participants younger than 5 years. For viral and antibody testing, 2 Oracol sponges per time point are required to ensure a sufficient volume of saliva. A third Oracol sponge will be collected per time point and placed in a tube pre-filled with 0.5 ml DEPC water with 50% glycerol. Saliva will then be stored in the home freezer inside a sealable plastic bag until picked up by a courier within 14-21 days after collection.
At day 7, NP and OP swabs will be collected from index cases and household members by trained research nurses using Copan Eswabs, and immediately placed in 1.0 ml Amies medium during a home visit. Nurses will wear appropriate protective gear (gloves, mask, coat, glasses).
At day 42, a capillary blood sample will be obtained by a finger prick from index cases and household members using a Microtainer safety lancet, after which 10 drops of blood will be collected in a microtube. Participants can collect the blood sample themselves, but a research nurse will assist if needed.

Transport and storage:
Samples will be transported to the laboratory by a courier within 14-21 days from collection in a cold chain. After transport to the laboratory, samples with 3 ml of raw saliva will be thawed and split into two aliquots: one for PCR analysis of respiratory viruses (2 ml) and one (1 ml)

for antibody detection. The raw Oracol sponges will be centrifuged and then split into the same two aliquots. The saliva samples with glycerol will be stored frozen at -80°C until analysis. By contrast, NP and OP samples will directly be analyzed for presence of SARS-CoV-2 by PCR following transport to the laboratory. The remaining material will be stored at -80°C. Blood samples obtained on day 42 will be transported at 4°C and spun down upon arrival at the laboratory, after which they are also stored at -80°C.

Questionnaires and clinical data:

An online questionnaire will be obtained at the first contact, to record baseline data from the patient and from household members. The baseline questionnaire contains questions on on household composition, household characteristics (number of bedrooms, sharing of bed(room)s, number of toilets, presence of washing basins and presence of pets), smoking, medical history, medication use, vaccination status, education level, profession, recent contact with people outside of the household (with and without respiratory complaints/proven COVID-19) and recent travel (in- and outside The Netherlands). Next, short online questionnaires to assess respiratory symptoms in the index patient after hospital discharge and household members will be filled out the nights before the predefined time points for saliva collection (day 1, 2 , 3 , 5, 7, 10, 14, 18, 21, 28, 35, 42 from the positive test result). Participants will also indicate whether they visited the general practitioner (and if yes, the date and name of the general practitioner) or the hospital (and if yes, the date and name of the hospital) in these questionnaires, so that medical records can be requested when applicable. Clinical data will then be extracted from the medical records. We will record routine care data such as clinical signs and symptoms, results from viral PCR or culture, results from routine blood tests, results of blood culture and chest X-ray and CT scan if performed, treatment with antibiotics, oxygen or other medications initiated for COVID-19, complications and duration of hospitalization when applicable.

Finally, at the end of the study, we will send out a short questionnaire with questions on how participants experienced the study and all procedures, leaving room for suggestions on improvements.

Laboratory methods:

SARS-CoV-2:

SARS-CoV-2 presence and viral loads will be evaluated. Nucleic acid extraction is conducted via MagNA Pure96 (Roche) and reverse transcriptase quantitative PCR is performed on Lightcycler 480 system (Roche) targeting the E gene according to the standard operating procedure for SARS-CoV-2 diagnostics.

Viral co-infections:

Presence of other respiratory viruses and bacteria will be assessed in saliva using the RespiFinder® 2Smart (PathoFinder B.V., Maastricht, Nederland) multiplex PCR. This assay allows detection of 18 viruses (influenza A en B, RSV A en B, rhinovirus/enterovirus, adenovirus, hMPV, influenza A (H1N1) pdm09, parainfluenza type 1, 2, 3, and bocavirus, coronaviruses NL63/HKU1, 229E, and OC43). Also 4 bacterial respiratory pathogens (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumoniae,* and *Bordetella pertussis*) are included. This analysis will be performed once for all index cases and once for household members who develop respiratory symptoms during follow-up, but tested negative for SARS-CoV-2.

Antibodies:

Salivary SARS-CoV-2 specific antibodies (immunoglobulins IgM, IgA and IgG) will be measured to assess the development of (mucosal) immunity over time once per week. SARS-CoV-2 specific antibodies (immunoglobulins IgG, IgA and IgM) will also be measured in serum collected at the end of the study period after 6 weeks. Antibodies will be measured using microarray to quantify the different types of immunoglobulins separately, and by Wantai ELISA to quantify IgM and the total immunoglobulin level (Silva et al., 2017). Both methods are complementary and will be used to obtain more insight and obtain maximum sensitivity.

Bacterial co-detection:

Quantitative pathogen-specific PCR for *Streptococcus pneumoniae* (piaB & lytA), *Haemophilus influenzae* (hpd), *Neisseria meningitidis* (metA & ctrA), *Staphylococcus aureus* (nuc), and *Streptococcus pyogenes* (spy1258) will be performed on saliva, NP and OP samples. For comparison of overall bacterial density and normalization of bacterial abundances, universal 16S qPCR and CRP (human DNA) qPCR will be performed. For identification and quantification of *Streptococcus pneumoniae,* an additional culture-enrichment step will be performed using the saliva, NP and OP samples containing DEPC water with 50% glycerol.

Microbiome and mycobiome:

Bacterial and fungal DNA will be isolated and quantified using qPCR of the 16S ribosomal RNA (rRNA) gene for bacteria and the internal transcribed spacer (ITS) for fungi. Next, bacterial profiles will be generated by amplification and sequencing of the 16S rRNA gene on an Illumina sequencing platform. Fungal profiles will be generated by ITS sequencing.

All collected and processed, remaining sample materials will be stored in the -80°C freezer at the study site and available for additional virological and serological studies, when applicable.

d. 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.

e. Follow-up of subjects withdrawn from treatment

If subjects want to leave the study, permission of the subject will be asked for processing the obtained material

f. Premature termination of the study

In case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination. Samples collected until that point will be analysed, upon permission of the participant.

9. SAFETY REPORTING

a. Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

b. AEs, SAEs and SUSARs

iv. Adverse events (AEs) and Serious adverse events (SAEs)

Every SAE directly related to any of the sampling interventions (capillary puncture or nasopharyngeal- oral swab) will be registered. Only SAEs will be registered as this is a non-interventional, low risk, observational study. Expected AEs directly related to one of the interventions (for example a nose bleed after a nasopharyngeal swab or bruise after a blood test) will not be registered.

c. Data Safety Monitoring Board (DSMB) / Safety Committee

No Data Safety Monitoring Board will be established.

10. STATISTICAL ANALYSIS

a. Primary study parameter(s)

To address the primary study objectives, the following analyses will at least be performed:

- To assess if SARS-CoV-2 diagnostic testing in saliva samples may replace NP and OP swabbing, sensitivity of testing for SARS-CoV-2 in saliva compared to routine diagnostics using NP and OP swabs as well as NP and OP swabs collected on day 7 of the study, will be computed. Saliva samples from day 1 will be compared with the primary diagnostic swab, and saliva samples from day 7 will be compared with the NP and OP swab from day 7. 95% Confidence intervals will be calculated using the Score confidence interval (when the proportion is in the range [5%-95%]), or with the Exact confidence interval. Concordance will also be tested using McNemar's test.
- To address sensitivity of SARS-CoV-2 tracing in potentially a- or pre-symptomatic household members, detection of SARS-CoV-2 will be temporally evaluated in relation to (the start of) clinical symptoms as documented in the questionnaire;
- Presence of SARS-CoV-2 and viral load over time in index patients and household members will be related to the presence and severity of clinical symptoms as documented in the questionnaire, using linear mixed effect models to account for repeated measures, including both individual subject and household as a random effect;
- To assess whether cases and household members develop immunity against SARS-CoV-2, the emergence of specific salivary antibodies will be described and compared between index SARS-CoV-2 positive subjects and household controls. Concordance of serum and salivary antibody levels and change over time will be evaluated using Wilcoxon signed rank test (matched samples) and Mann-Whitney U-test (between groups), and linear regression models.

b. Secondary study parameter(s)

To address the secondary objectives, the following analyses will at least be performed:

- Detection of other respiratory viruses will be related to presence and severity of clinical symptoms;
- Detection of other respiratory bacteria over time in SARS-CoV-2 positive index subjects and household members will be related to presence and severity of clinical symptoms.

c. Other study parameters

To address the exploratory objectives:

- The composition of the upper respiratory microbiome and mycobiome will be compared between SARS-CoV-2 positive subjects and SARS-CoV-2 negative subjects. Finally, the composition of the microbiome and/or mycobiome will be related to severity of COVID-19.

The final dataset will be published in an open-access data repository (Dataverse) and will be shared with collaborating partners in the project for research purposes and shared with the wider scientific community upon COVID 19 or other FAIR data point.

d. Interim analysis (if applicable)

Not applicable

11. ETHICAL CONSIDERATIONS

a. Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines as code of conduct involving minors.

b. Recruitment and consent

We will recruit patients with COVID-19 up to 65 years old with a household that consists of at least 3 people including the index case. We will recruit at different sites, through the Spaarne Gasthuis in Haarlem, the general practitioners (GP) in the region of Hoofddorp and Haarlem, and the municipal health services Kennemerland. At all sites, recruitment material will be present (leaflets and/or posters). Eligible subjects with laboratory-confirmed COVID-19 will be asked if they are willing to participate when the positive test result is communicated to them as a part of the routine diagnostic procedures. This will be done by staff from the Spaarne Gasthuis or from the municipal health services Kennemerland, or by the potential participant's GP, depending on who requested the test. If they agree, contact details from potential participants will be communicated to the study team by phone or secure e-mail. Next, on the same day if possible, the study team will approach the potential participants, inform them about the study and answer questions, and send them the patient information leaflet with informed consent form via e-mail. This corresponds to day 0 of the study. When they are interested to participate, study staff will drop off material and obtain written informed consent from them and at least 2 of their household members on the next day (day 1, at least 12 hours later). At this time, study staff may again answer questions. Because of COVID-19, study staff will be required to keep a distance and will not immediately sign the form. Instead, staff will take the signed forms and keep them in a sealed bag for 72 hours. After this time, study staff will also sign the form and return a copy to the study participants. For children below the age of 12 years, both parents/caregivers need to give written consent. For children 12-15 years of age, both parents/caregivers and the child need to give written consent.

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

The code of conduct of the Dutch Society of Paediatrics (NVK) is applicable to the participants and is also referred to in the informed consent letter.

d. Benefits and risks assessment, group relatedness

The burden of participating in this study is low. Blood collection will take place only once and will be performed by a finger prick, so discomfort is transient and limited to a minimum and

the procedure holds no risk for the participant. Infection occurs very rarely. NP and OP swabs are also collected only once. NP swabbing gives mild discomfort, may induce sneezing, and may give a nosebleed in rare cases. OP swabbing also gives mild discomfort and may induce a gagging reflex. Saliva collection holds no risk. Saliva sampling will be repeated in total 10 times for SARS-CoV-2 positive subjects and household members, but represents no burden also when repeated. Participants will also be required to complete a total of 12 questionnaires, which is expected to take 12x5 minutes maximum per person in the household. Study staff will drop off required sampling material at participant homes, keeping at least 1.5 meters distance. At the end of follow-up, study staff will also pick up all the samples that were collected at home, taking necessary precautions to ensure their safety.

There is no clear clinical benefit for the subjects participating in the study. The study physician will inform participants about the results on SARS-CoV-2 testing from the NP and OP swab obtained on day 7 by phone as soon as results are available. Testing of these swabs will be performed immediately. However, study participants should still follow the advice from the municipal health services regarding testing for SARS-CoV-2. At the end of the study, the study physician will also inform participants on the results from the saliva samples. This will be done several weeks after the last collection moment, when all samples have been analyzed. Study procedures will be evaluated by all participants, leaving room for suggestions. Results regarding the secondary and exploratory objectives (presence of other respiratory viruses, bacteria, the microbiome and mycobiome) will not be communicated to the participants. These results have no clinical consequences for the participants.

e. Compensation for injury

The sponsor/investigator has requested the METC for exemption of liability insurance.

f. Incentives (if applicable)

Not applicable

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

a. Handling and storage of data and documents

To guarantee privacy, data will be coded; every participant will receive a participant-specific identification code (a study-specific identifier followed by a household-specific number and a participant-specific identifier). All patient materials (saliva, nose- and throat swabs, blood) will be stored in a local freezer at the Spaarne Gasthuis coded with the participant-specific identification code. Study documents will be maintained in a locked room with controlled access in the Spaarne Gasthuis. Personal data that may identify participants (name, date of birth, address) will only be accessible by the study personnel; only the principal investigators and the coordinating investigator from the Spaarne Gasthuis have access to these codes. Data will be collected using Research Manager: a database that we have an agreement with and that complies with national data security and privacy standards. All data and patient materials will be stored up to 15 years.

The final, coded dataset will be shared with collaborating partners in the project (RIVM, Streeklab Haarlem) for research purposes. The final, coded dataset will also be published in an open-access data repository (DataverseNL) and shared with the wider scientific community upon COVID-19.This is in accordance with requirements from ZonMw that data should be findable, accessible, interoperable and reusable (FAIR). All data will be coded, so privacy will be guaranteed.

b. Monitoring and Quality Assurance

Data will be controlled for quality (accuracy) by monitoring. Monitoring will be arranged in accordance to the monitoring guidelines based on risk level, in case of children one level higher so slightly more risk than negligible risk. Data and body material will be processed in accordance with the General Data Protection Regulation (GDPR).

c. Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

d. Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the starting date of the study, the date of

inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

e. Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

f. Public disclosure and publication policy

Results including negative results will be published unreservedly by the investigator in international literature.

14. REFERENCES

Azzi, L., Baj, A., Alberio, T., Lualdi, M., Veronesi, G., Carcano, G., … Fasano, M. (2020). Rapid Salivary Test Suitable for a Mass Screening Program To Detect Sars-Cov-2: a Diagnostic Accuracy Study. *Journal of Infection*. https://doi.org/10.1016/j.jinf.2020.06.042

Azzi, L., Carcano, G., Gianfagna, F., Grossi, P., Gasperina, D. D., Genoni, A., … Baj, A. (2020). Saliva is a reliable tool to detect SARS-CoV-2. *Journal of Infection*, *81*, e45–e50. https://doi.org/10.1016/j.jinf.2020.04.005

Brown, L. D., Cai, T. T. & DasGupta, A. (2001). Interval estimation for a binomial proportion. *Statistical Science*, *16*(2), 101-133

Kinloch, N. N., Ritchie, G., Brumme, C. J., Dong, W., Dong, W., Lawson, T., … Brumme, Z. L. (2020). Suboptimal biological sampling as a probable cause of false-negative COVID-19 diagnostic test results Natalie. *The Journal of Infectious Diseases*, jiaa370.

Krone, C. L., Wyllie, A. L., van Beek, J., Rots, N. Y., Oja, A. E., Chu, M. L. J. N., … Trzciński, K. (2015). Carriage of Streptococcus pneumoniae in aged adults with influenza-like-illness. *PLoS ONE*, *10*(3), 1–15. https://doi.org/10.1371/journal.pone.0119875

Randad, P. R., Pisanic, N., Kruczynski, K., Manabe, Y. C., Thomas, D., Pekosz, A., … Heaney, C. D. (2020). COVID-19 serology at population scale: SARS-CoV-2-specific antibody responses in saliva. *MedRxiv*.

Rodrigues, F., Christensen, H., Morales-Aza, B., Sikora, P., Oliver, E., Lucidarme, J., … Finn, A. (2019). Viable Neisseria meningitidis is commonly present in saliva in healthy young adults: Non- invasive sampling and enhanced sensitivity of detection in a follow-up carriage study in Portuguese students. *PLoS One*, *14*(2), e0209905.

Silva, T. I. de, Gould, V., Mohammed, N. I., Cope, 5 5.1.2e ., Zutt, I., … Tregoning, J. S. (2017). Comparison of mucosal lining fl uid sampling methods and in fl uenza-speci fi c IgA detection assays for use in human studies of influenza immunity. *Journal of Immunological Methods*, *449*, 1–7. https://doi.org/10.1016/j.jim.2017.06.008

To, K. K. W., Tsang, O. T. Y., Chik-Yan Yip, C., Chan, K. H., Wu, T. C., Chan, J. M. C., … Yuen, K. Y. (2020). Consistent detection of 2019 novel coronavirus in saliva. *Clinical Infectious Diseases*, ciaa149. https://doi.org/10.1093/cid/ciaa149

Williams, E., Bond, K., Zhang, B., Putland, M., & Williamson, D. A. (2020). Saliva as a non-invasive specimen for detection of SARS-CoV-2. *Journal of Clinical Microbiology*, *JCM.00776-*. https://doi.org/10.1128/JCM.00776-20

Wyllie, Anne L., Chu, M. L. J. N., Schellens, M. H. B., van Engelsdorp Gastelaars, J., Jansen, M. D., van der Ende, A., … Trzciński, K. (2014). Streptococcus pneumoniae in Saliva of Dutch Primary School Children. *PLoS ONE*, *9*(7), e102045. https://doi.org/10.1371/journal.pone.0102045

Wyllie, Anne Louise, Fournier, J., Casanovas-Massana, A., Campbell, M., Tokuyama, M., Vijayakumar, P., … Ko, A. I. (2020). Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. *MedRxiv*, (2), 2020.04.16.20067835. https://doi.org/10.1101/2020.04.16.20067835

Zhu, J., Guo, J., Xu, Y., & Chen, X. (2020). Viral dynamics of SARS-CoV-2 in saliva from infected patients. *Journal of Infection*. https://doi.org/10.1016/j.jinf.2020.06.059