

Onderzoeksprotocol

(voor aanvraag niet-WMO verklaring)

General data

Title	SARSLIVA: utility of saliva in diagnosis, detecting co-infections, and evaluating household transmission in COVID-19
Date	March 27, 2020
Version number	1
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Study data

Rationale	The novel coronavirus (SARS-CoV-2) disease has been declared a pandemic by the WHO (WHO 2020a). Associated with high morbidity and mortality, SARS-CoV-2 is genetically related to SARS-CoV-1 (severe acute respiratory syndrome-related coronavirus) the cause of the 2002-2004 SARS outbreak (WHO 2004), and to MERS-CoV (Middle Eastern respiratory syndrome-related coronavirus), which has been causing the ongoing MERS outbreak since its emergence in 2012. These outbreaks and previous influenza pandemics are largely shaping
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	our approach to tackling the current pandemic. Nonetheless, understanding risk factors for infection, transmission patterns and severity of clinical features remains limited and studies assessing epidemiological and clinical characteristics of cases are critical for our understanding of this virus and associated diseases. Like SARS-CoV-1, SARS-CoV-2 is able to enter human cells through the ACE2 cell receptor (Hoffmann et al. Cell 2020). ACE2 cell receptors are abundantly expressed among cells of the respiratory tract. Target cells of SARS-CoV-1 were reported to be ACE2(+) epithelial cells in the salivary gland ducts of rhesus macaques. While this has not yet been confirmed for SARS-CoV-2, shedding of viable SARS-CoV-2 has been reported in saliva (To et al. CID 2020) . As infection of the salivary glands may represent early target cells of SARS-CoV-1 (Liu et al J.Virol 2011), saliva may have an important role in (pre-symptomatic) transmission of SARS-CoV-2 via oral droplets, as it was estimated that pre-symptomatic transmission contributed to 48% (95%CI 32-67%) and 62% (95%CI 50-76%) of diagnosed COVID-19 cases in Singapore and in Tianjin, China, respectively (Tapiwa et al. medRxiv). Sample collection for SARS-CoV-2 diagnosis Similar to other respiratory viruses the ability of detecting this coronavirus will largely be dependent on the sample collected. The optimal specimen type for SARS-CoV-2 detection is yet to be determined. Currently the WHO recommends screening individuals for the SARS-CoV-2 virus with nucleic acid amplification tests (NAAT), such as reverse-transcription PCR (RT-PCR) (WHO 2020b, Zou et al. NEJM 2020). For ambulatory patients, the primary approach for collecting samples is via nasopharyngeal (NP) <u>and</u> oropharyngeal (OP) swabs <u>or</u> NP wash/NP aspirate (NPA). In patients with more severe respiratory symptoms, it is also recommended to test lower respiratory tract samples (sputum, aspirate, lavage) collected on presentation (WHO 2020b). On the top of these, WHO also recommends screening of asymptomatic contacts in health-care
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	associated outbreaks or when individuals had high-intensity contact with a COVID-19 case. For this, NP and OP swabs are also used for testing with NAAT (WHO 2020b). On top of this, WHO recommends to start testing all these individuals for SARS-CoV-2 antibodies once the immunological method(s) are validated and available (WHO 2020b). One of the samples currently missing from the WHO guidelines is saliva. Saliva could be the one sample that can fit the broadest possible range of purposes in COVID-19 diagnostics. Saliva can be tested (I) to detect and quantify the presence of SARS-CoV-2 in upper airways; (II) to detect the presence of SARS-CoV-2 antibodies; (III) to detect and quantify the presence of other etiological agents (bacteria and fungi) causing the secondary infections common in influenza-like-illness. Finally, saliva could be also used (IV) to determine the respiratory microbiota composition. Saliva has been reported to be equally (if not more) effective to NP swab diagnostics for a broad range of respiratory viruses (To et al. CMI 2019, Robinson et al. Clin.Infect.Dis. 2008, Niedrig et al. BMC Infect. Dis. 2016, To et al. Emerg. Microbes Infect. 2017). Most importantly, it was proved to be superior to tracheal wash in quantification coronavirus in the detection of 2002-2003 SARS-CoV-1 (Wang et al. Emerg.Infect.Dis. 2004). Furthermore, saliva has been proven to be highly informative when testing for the presence of SARS-CoV-2 in individuals diagnosed with COVID-19 (To et al. Clin.Infect.Dis. 2020). Saliva was also equally informative in serological (antibody) diagnostics of viral respiratory infection, but not specifically for coronaviruses (Niedrig et al. BMC Infect. Dis. 2016). However, there is currently no study that systematically compares directly NP and OP swabs with saliva for sensitivity of SARS-CoV-2 detection. Studies that compare saliva for sensitivity of SARS-CoV-2 antibody detection with or without the presence of SARS-CoV-2 are currently ongoing. WHO issues repeated calls to maximize testing for SARS-CoV-2 virus
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	and for antibodies, in order to determine how many people have been infected and who is still at risk of being infected and transmission patterns. This information is crucial to guide decisions and to monitor the impact of any interventions. To maximize testing of large cohorts of individuals, high throughput methods need to be in place. Among multiple advantages of saliva-based diagnostics, is simplicity of its collection. Saliva can be self-collected, therefore it is much easier to collect than any respiratory sample currently recommended for SARS-CoV-2 diagnostics (swabs or blood sample). Saliva can be obtained by participants spitting into a tube or saliva collection with a sponge or swab without requiring close contact between the patient/participant and healthcare worker. Unlike the procedure to obtain NP or OP swabs, collection of saliva is unlikely to induce coughing. All of this, reduces risk of spread of a virus. Saliva can be collected at a much lower cost (cost is reduced by not using collection kits) (Kim et al. JCM 2016). If required, saliva still can be bundled for processing, if necessary, with other samples (e.g. NP swab) (Kim et al. JCM 2016). In general, conditions of processing, including transportation and sample storage, are similar or even simpler compared with conditions required for corresponding procedures for other sample types given the right transport and storage media are in place. Characterizing interactions between SARS-CoV-2 and microbial abundance Common symptoms at onset of illness caused by the SARS-CoV-2 virus include fever, cough, and fatigue or myalgia (Zhou et al. Lancet 2020, Ding et al. JMV 2020) and are consistent with symptoms of with influenza like illness (ILI) (Pel, Huisarts en Wetenschap 1965). Among the clinical features of COVID-19 is the induction of pro-inflammatory cytokines (IL1B, IFNγ, IP10, and MCP1) which can impair immunological control of bacterial growth and predispose patients with COVID-19 to secondary bacterial infection (Huang et al. Lancet
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	2020, Bosch et al. PLoS Pathog. 2013). In line with this, sepsis has been reported to be a common complication of COVID-19 (Zou et al. Lancet 2020). As such, clinical management of COVID-19 can include antibiotics to reduce likelihood of coinfection by <i>Streptococcus pneumoniae</i> and <i>Staphylococcus aureus</i> (Zhang et al. Lancet Resp. Med. 2020). Epidemiological studies suggest a strong link between viral respiratory infections manifested by influenza-like illness (and) pneumococcal disease (Li et al. BMJ Open 2018). It is assumed that perturbations in the URT by viruses and the subsequent host responses may disrupt the balanced host-commensal relationship, allowing outgrowth of certain bacterial pathogens (Bosch et al. PLoS Pathog. 2013). Similar to COVID-19, the burden of pneumococcal disease also peaks in older adults (Fleming and Elliot Vaccine 2005, Kristensen et al. Vaccine 2016). Saliva not only can be used for viral but also bacterial diagnostics (Wyllie et al. PLoS One 2014, Wyllie et al. SciRep. 2016b, Krone et al. Int J Mol Sci. 2016). Saliva has been proven superior to both NP or OP swabs in detection of the presence of <i>S. pneumoniae</i> in upper airways, a leading bacterial cause of secondary infection following influenza-like illness (Krone et al. PLoS One 2015). For this, saliva samples collected for viral diagnostic could tested along also for respiratory bacterial pathogens. The entire microbial community including bacteria, viruses and fungi, present in the different niches of the respiratory tract together represent the respiratory microbiota. Profiling of respiratory microbiota is commonly done by next generation sequencing methods, including 16S rRNA gene sequencing for the bacterial microbiome and internal transcribed spacer (ITS) sequencing for the mycobiome (fungi). In both children and adults, during a (lower) respiratory tract infection, the bacterial microbiome of the upper respiratory tract is strongly different compared to healthy controls (Man et al. Lancet Resp. Med. 2019, de Steenhuijsen Piters et al. ISME
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	2015). Moreover, the composition of the upper respiratory tract microbiome is related to susceptibility to and severity of respiratory infections, which may imply causal involvement in infection pathogenesis (Bosch et al. Am. J. Respir. Crit. Care Med. 2017, de Steenhuijsen Piters Am. J. Respir. Crit. Care Med. 2016). Fungi (i.e. the mycobiome) likely also contribute significantly to respiratory disease, but have not yet been studied extensively in relation to respiratory infections (Nguyen et al. Front. Microbiol. 2015). Whether the composition of respiratory microbial communities also (partially) explain why some individuals with COVID-19 develop more severe symptoms than others remains open to investigation. Characterizing the host response against SARS-CoV-2/COVID-19 For profiling immune responses, salivary antibody levels (immunoglobulin G) were previously shown to correlate well to serum levels, though not for coronaviruses (Rodenburg et al. PLoS One 2012 Barnes et al. Scand. J. Immunol. 2016). Immunoglobulin G in saliva is likely derived from the blood circulation by passive transudation (Brandtzaeg J. Oral. Microbiol. 2013). Saliva may therefore represent a valid, non-invasive and easily accessible alternative to blood for monitoring humoral immune responses, also against SARS-CoV-2. REFERENCES Bao et al. bioRxiv Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. Barnes et al. Scan. J. Immunol. 2016 Salivary and Serum Antibody Response Against Neisseria meningitidis After Vaccination With Conjugate Polysaccharide Vaccines in Ethiopian Volunteers. Bosch et al. Am. J. Respir. Crit. Care Med. 2017 Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health Consequences. A Prospective Cohort Study. Brandtzaeg J. Oral Microbiol. 2013 Secretory immunity with special reference to the oral cavity Bosch et al. PLoS Pathog. 2013 Viral and bacterial interactions in the
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	respiratory virus by a point-of-care molecular assay: a diagnostic validity study. To et al. Clin.Infect.Dis. 2020 Consistent Detection of 2019 Novel Coronavirus in Saliva. Zhang et al. Lancet Resp. Med. 2020 Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics Zhou et al. Lancet 2020 Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Zou et al. NEJM 2020 SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. Zou et al. Lancet 2020 Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study respiratory microbiome Wang et al. bioRxiv A human monoclonal antibody blocking SARS-CoV-2 infection. Wang et al. Emerg.Infect.Dis. 2004 Detection of SARS-associated Coronavirus in Throat Wash and Saliva in Early Diagnosis WHO 2004 Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. WHO 2020a Coronavirus disease (COVID-19) Pandemic. WHO 2020b Laboratory testing for coronavirus (COVID-19) in suspected human cases. Interim guidance 19 March 2020. (as on 22-March-2020) Wyllie et al. PLoS One 2014 Streptococcus pneumoniae in saliva of Dutch primary school children. Wyllie et al. Sci.Rep. 2016a Molecular surveillance of nasopharyngeal carriage of Streptococcus pneumoniae in children vaccinated with conjugated polysaccharide pneumococcal vaccines. Wyllie et al. SciRep. 2016b Molecular surveillance on Streptococcus pneumoniae carriage in non-elderly adults; little evidence for pneumococcal circulation independent from the reservoir in children.
Objectives	We propose a study with the following objectives:

	The <u>primary objective</u> of this study is to investigate if SARS-CoV-2 detection in saliva is comparable or even superior to nasopharyngeal (NP) and oropharyngeal (OP) swabs. <u>Secondary objectives</u> of this study are: a) To compare viral load of SARS-CoV-2 in saliva, NP and OP swabs; b) To assess salivary viral load of SARS-CoV-2 in saliva over time, in relation to the course of disease; c) To investigate transmission and salivary viral load of SARS-CoV-2 in household members, and the course of potential COVID-19 disease in household members during follow-up of 42 days; d) To investigate whether patients and (potentially asymptomatic) household members develop salivary antibodies against SARS-CoV-2. <u>Exploratory objectives</u> are: a) To explore age-related differences in sensitivity of detection of SARS-CoV-2 in saliva compared to NP and OP swabs. b) To explore whether viral and bacterial co-infections as well as the composition of the upper respiratory tract microbiome and mycobiome are associated with (severity of) COVID-19.
Study design	This prospective cohort study will investigate whether saliva is as good as or better than NP and OP swabs to detect SARS-CoV-2 in children and adults. Next to that, it will assess potential viral and bacterial co-infections in (severe) COVID-19, progression of disease symptoms and viral shedding, transmission to household contacts, and development of immunity against SARS-CoV-2, including in (potentially asymptomatic) household contacts. Children and adults up to 60 years old who visit the special coronavirus department for patients suspected of COVID-19 of the Spaarne Gasthuis in Haarlem and are tested for SARS-CoV-2, will be

	recruited. From them, additional samples (see below: clinical samples) will be collected. Subjects who test positive for SARS-CoV-2 by routine diagnostics and their household members will then be recruited for follow-up. Follow-up of subjects and household members will be 6 weeks in total. In case subjects are admitted to the hospital, saliva sampling will be repeated by the treating physician at predefined timepoints (see below: clinical samples) until hospital discharge and information about the course of disease will be obtained from medical records (see below: clinical data during hospital admission). When subjects go home, saliva will be collected by themselves or a household member and information about the course of disease will be obtained from questionnaires (see below: questionnaires). <i>Clinical samples:</i> From all subjects, one additional saliva swab will be collected in the special coronavirus department, in addition to routinely collected NP and OP swabs. From subjects who tested positive for SARS-CoV-2, saliva will also be collected on day 3, day 5, day 7, day 14, day 30, and day 42 from presentation at the hospital. Furthermore, saliva will also be collected from household members of SARS-CoV-2 positive subjects on the day of hospital presentation of the index COVID-19 patient, and on day 3, day 5, day 7, day 14, day 30, and day 42. Because of the high risk of infection for study personnel and the lack of protective materials, collection of saliva at home will be performed by the participants themselves, following detailed instructions. <i>Clinical data during hospital admission:</i> For subjects who are admitted to the hospital for COVID-19, we will record routine care data such as patient characteristics, time of disease onset, clinical signs and symptoms, results from viral PCR for other respiratory viruses, inflammatory parameters (leucocytes, differential count, CRP), results of blood culture and chest X-ray if performed, treatment complications and duration of hospitalization will be documented from all hospitalized SARS-CoV-2 infected
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subjects. These data will be obtained from electronic medical records. <i>Questionnaire:</i> An online questionnaire will be obtained from subjects who test positive for SARS-CoV-2 on the composition of the household including age of household members and the presence of pets. From all household members, information on smoking habits, allergies, medical history and medication use including recent antibiotic use will be asked. Further online questionnaires will be used to investigate the course of disease in SARS-CoV-2 positive subjects who are not admitted to the hospital, or after hospital discharge, as well as symptoms in household members. The table below provides an overview of the timing of sample collection and the questionnaires:			
	SARS-CoV-2 positive subjects (n=40)	Household members of SARS-CoV-2 positive subjects	SARS-CoV-2 negative subjects (n=80)
Day 1	Saliva + clinical data (NP + OP are routine care)	Saliva + questionnaire	Saliva (NP + OP are routine care)
Day 3	Saliva + questionnaire/ clinical data	Saliva + questionnaire	
Day 5	Saliva + questionnaire/ clinical data	Saliva + questionnaire	
Day 7	Saliva + questionnaire/ clinical data	Saliva + questionnaire	
Day 14	Saliva +	Saliva +	

		questionnaire/ clinical data	questionnaire	
	Day 30	Saliva + questionnaire/ clinical data	Saliva + questionnaire	
	Day 42	2x Saliva + questionnaire	2x Saliva + questionnaire	
Table 1: Overview of sampling moments and questionnaires				
Study population	Children (<18 years old) and adults (18-60 years old) who are tested for SARS-CoV-2 at the corona department of the Spaarne Gasthuis at Haarlem.			
Inclusion criteria	In order to be eligible to participate in this study, a subject must meet all of the following criteria: - Tested at the corona department for a suspected infection with SARS-CoV-2; and - Between 0-60 years old.			
Exclusion criteria	A potential subject who meets any of the following criteria will be excluded from participation in this study: - Language barrier; or - When a patient is too ill to give informed consent.			
Sample size	In order to address the primary objective, a total of 40 SARS-CoV-2 positive subjects will be required, as well as 80 SARS-CoV-2 negative subjects. In order to be able to say something about the pediatric population, we will strive to include at least 10 SARS-CoV-2 positive and 20 SARS-CoV-2 negative children. Given the rapid spread of the COVID-19 pandemic, we expect to be able to include a sufficient number of adult subjects. Since children more often remain asymptomatic, or only have mild disease symptoms, they are likely to present to the corona department less often. This sample size is based on previous reports of tests for other infectious agents yielding comparable results in saliva as in NP and OP swabs (Kim et al. JCM 2016, To et al. Emerg. Micobes Infect. 2016, To			

	et al. CMI 2019, To et al. Clin. Infect. Dis. 2020.). References <u>KIM ET AL. JCM 2016</u> COMPARISON BETWEEN SALIVA AND NASOPHARYNGEAL SWAB SPECIMENS FOR DETECTION OF RESPIRATORY VIRUSES BY MULTIPLEX REVERSE TRANSCRIPTION-PCR. <u>TO ET AL. EMERG. MICROBES INFECT. 2017</u> ADDITIONAL MOLECULAR TESTING OF SALIVA SPECIMENS IMPROVES THE DETECTION OF RESPIRATORY VIRUSES <u>TO ET AL. CMI 2019</u> SALIVA AS A DIAGNOSTIC SPECIMEN FOR TESTING RESPIRATORY VIRUS BY A POINT-OF-CARE MOLECULAR ASSAY: A DIAGNOSTIC VALIDITY STUDY. <u>To et al. Clin.Infect.Dis. 2020</u> Consistent Detection of 2019 Novel Coronavirus in Saliva.
Recruitment	Patients suspected of COVID-19 will first present at the special corona department. There, <i>oral</i> consent will first be obtained to take additional an additional saliva sample, next to routinely collected NP and OP swabs. After the SARS-CoV-2 result is available, and within 72 hours from presentation at the hospital, the treating physician or study staff will obtain written informed consent to process and store these additional samples, and will ask SARS-CoV-2 positive subjects for additional consent to collect follow-up samples and send out questionnaires after hospital discharge (see Table 1 and below: sample collection). For pediatric patients <12 years old, parents will be asked to give oral and written consent. For pediatric patients 12-15 years old, both parents and the patient will be asked to give oral and written consent. Pediatric patients >15 years old and adult patients will be asked to give written and oral consent themselves. For subjects who tested positive for SARS-CoV-2, household members will also be contacted by study staff and will be asked to give oral and written consent. For household members <12 years old, parents will be asked to give written informed consent. For household members 12-15 years old, both the child and the parents will be asked to give written informed consent.
Study procedures	<u>Sample collection:</u> NP and OP swabs will be collected by trained physicians, and will be

	immediately placed in Amies medium and transported to the Streeklab Haarlem. After testing for the presence of SARS-CoV-2 and other respiratory viruses, the remainder of the material will be stored at -80 °C. Saliva samples will be obtained with a sterile swab and immediately placed in tubes with RNA-protect Bacteria Reagent. Saliva obtained at hospital presentation will immediately be transported to the Streeklab Haarlem, and after testing for SARS-CoV-2 and other respiratory viruses, the remainder of the material will be stored at -80°C. Saliva obtained during follow-up from SARS-CoV-2 positive subjects and from household members will first be stored at -20°C before transport to the Streeklab Haarlem, where they will be stored at -80°C. Transport of follow-up samples from the corona ward to the Streeklab Haarlem will take place within 72 hours. Samples obtained at home will be picked up by study staff wearing appropriate protective gear (gloves, mask, coat) within 14 days. At day 42, 1 additional saliva sample will be collected from SARS-CoV-2 positive subjects and household members using 'lollypop'-sponges during a home visit, and pushed into EDTA tubes containing protease inhibitors using a sterile syringe. These samples will immediately be frozen on dry ice and stored at -80°C. All samples will need to be disinfected before leaving the hospital room or the home. For further analyses, all samples will be transported to the National Institute of Public Health and the Environment (RIVM) on dry ice, where they will again be stored at -80°C until further analyses. <u>Questionnaires:</u> A questionnaire will be obtained at the first contact, to record baseline data from the patient and from household members (including e.g. age, gender, allergies, smoking, medical history, etc.). Next, questionnaires to assess respiratory symptoms in the index patient after hospital discharge and household members will be filled out at predefined timepoints (day 1, day 3, day 5, day 7, day 14, day 30 and day 42 from hospital presentation).
Standard of care	Not applicable. Collection of samples will not interfere with nor add to

	the standard of care.
Study parameters	The primary study parameter is the concordance of testing for SARS-CoV-2 between saliva and routine diagnostics performed on NP or OP swabs, or a combination of the two. Presence of SARS-CoV-2 in saliva will be determined by quantitative Real Time PCR at the Streeklab Haarlem.
Study end points	To address the secondary objectives, the following will be evaluated: - Severity of COVID-19 will be assessed using data from medical records and from questionnaires. Indicators of disease severity include duration of hospitalization, need for oxygen support, transfer to the intensive care unit, duration of symptoms, etc. - Viral load of SARS-CoV-2 in saliva, NP swabs and OP swabs will be assessed by quantitative PCR at the RIVM and related to disease severity; - Viral co-infections (i.e. other respiratory viruses) will be assessed in NP swabs, OP swabs and saliva by conventional multiplex PCR at the Streeklab Haarlem; - Bacterial co-infections/colonization will be assessed in saliva by quantitative pathogen-specific PCR for <i>Streptococcus pneumoniae</i> (<i>piaB</i> & <i>lytA</i>), <i>Haemophilus influenzae</i> (<i>hpd</i>), <i>Neisseria meningitidis</i> (<i>meta</i> & <i>ctrA</i>), <i>Staphylococcus aureus</i> (<i>nuc</i>), and <i>Streptococcus pyogenes</i> (<i>spy1258</i>) at the RIVM. For comparison of overall bacterial density and normalization of bacterial abundances, universal 16S qPCR and <i>CRP</i> (human DNA) qPCR can be performed. Age-matched asymptomatic controls will be included for comparison; - The NP, OP and salivary microbiome will be assessed using 16S ribosomal RNA gene sequencing at the RIVM. Age-matched asymptomatic controls will be included for comparison; - The NP, OP and salivary mycobiome will be assessed using Internal transcribed spacer (ITS) sequencing at the RIVM. Age-matched asymptomatic controls will be included for comparison; - Antibodies (immunoglobulin A, G, M) against SARS-CoV-2 in

	saliva will be assessed using a multiplex immunoassay with Bio-Plex 100/200 at the RIVM.
Statistical analysis	To assess if SARS-CoV-2 diagnostics could also be performed in saliva samples, sensitivity, specificity, positive predictive value and negative predictive value of testing for SARS-CoV-2 in saliva compared to NP and OP swabs will be computed using 2x2 contingency tables. 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 both Spearman Rank test and McNemar's test. To address the secondary objectives, the following analyses will at least be performed: - Viral load of SARS-CoV-2 will be calculated from Ct-values from quantitative PCR. This will be compared between saliva, NP swabs and OP swabs; - Viral load of SARS-CoV-2 in saliva over time will be related to parameters indicating severity of COVID-19. To this end, patients will be dichotomized according to disease severity (e.g. short vs. long hospitalization duration) and viral load will be compared between two groups; - To address transmission of SARS-CoV-2 to household members and development of symptoms in household members, detection of SARS-CoV-2 will be temporally evaluated in relation to respiratory symptoms as documented by the questionnaire; - To assess whether cases and household members develop immunity against SARS-CoV-2, salivary antibody levels against the virus at day 42 will be described and compared between Index SARS-CoV-2 positive subjects and household controls. To address the exploratory objectives: - The analyses described above will be performed for children and adults separately, if possible;

	- Detection of other respiratory viruses and bacteria will be compared between SARS-CoV-2 positive subjects and matched asymptomatic controls and SARS-CoV-2 negative subjects. Viral and bacterial co-detection will also be related to parameters indicating severity of COVID-19; - The composition of the NP and OP microbiome and mycobiome will be compared between SARS-CoV-2 positive subjects and SARS-CoV-2 negative subjects. We will further compare these to age- and gender-matched healthy controls from the PIENTER3 study. The PIENTER3 study is a population-wide Dutch cross-sectional study including individuals 0-80 years old, from whom NP and OP swabs were also taken. Finally, the composition of the microbiome and/or mycobiome will be related to severity of COVID-19.
Burden on participants	The burden of participating in this study is minimal. Saliva collection holds no risk (see below: risks for the participant). Saliva sampling will be repeated in total 7 times for SARS-CoV-2 positive subjects and household members, but represents no burden also when repeated. (Household members) of SARS-CoV-2 positive subjects will also be required to complete a total of 7 questionnaires, which is expected to take 7x20 minutes maximum. No additional visits to the hospital are required. If necessary, study staff will drop off required sampling material at participant homes. At the end of follow-up, study staff will also pick up all the samples that were collected at home.
Risks for participants	Participation in this study holds no additional risk than negligible risk. Saliva sampling is non-invasive and generally accepted as fully safe. No side effects are to be expected from collection of saliva samples. We will follow the code of conduct relating to expressions of objection by minors participating in medical research, as stated by the CCMO.
Benefits of participating in the study	Participation in this study holds no benefits.
Disadvantages of participating in the study	Participation in this study holds no disadvantages.

Reimbursement	Participants will not receive any form of compensation.
Administrative aspects	To guarantee privacy, data will be coded; every participant will receive a participant-specific identification code. All patient materials will be stored coded with the participant-specific identification code. Study documents will be maintained in a locked room with controlled access. Personal data from participants will only be accessible by the study personnel. Only the principal investigators and the coordinating investigator have access to these codes. All data and samples will be stored up to 15 years.
Publication and amendments	All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor. Results will be published anonymously by the investigator in international literature.
Other points of consideration for METc	