National Institute for public Health
and the Environment
Ministry of Health, Welfare and Sport

Use off frequent saliva sampling

5.1.2e

On behalf of
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UMCU

IDS VIR meeting| 23-03-2021

SARSLIVA - Use off frequent saliva sampling to measure household transmission of SARS-CoV-2

ZonMw

SARSLIVA: Utility of saliva in diagnosis for wide scale testing, including viral and antibody detection in pre- and asymptomatic persons and follow-up of infections in COVID-19 patient; a household study

Nasopharyngeal swabs versus use of Saliva

Nasopharyngeal swabs are currently the golden standard for SARS-CoV-2 detection (and sequencing)
Advantages:
- Very sensitive
- Standardized processing in laboratories

Disadvantages
- Trained healthcare professionals
- Protective equipment
- Possible discomfort

In contrast, collection of saliva is easy by self-sampling, causes no discomfort and this way avoids necessity of trained health care workers

## Nasopharyngeal swabs versus use of Saliva

Is Saliva (Oral fluid) an option?

In a direct comparison between 38 paired nasopharyngeal and saliva samples from COVID-19 patients Wyllie *et al*. found comparable or higher sensitivity and similar or higher SARS-CoV-2 loads in saliva compared to nasopharyngeal swabs

Within the RIVM FFX study, we found slightly lower sensitivity, but mostly when Ct values were rather high in the nasopharyngeal swabs (*final analyses still in progress*, 5.1.2e)
→ Good alternative, especially in children.

Specimen collection – oracol sponge
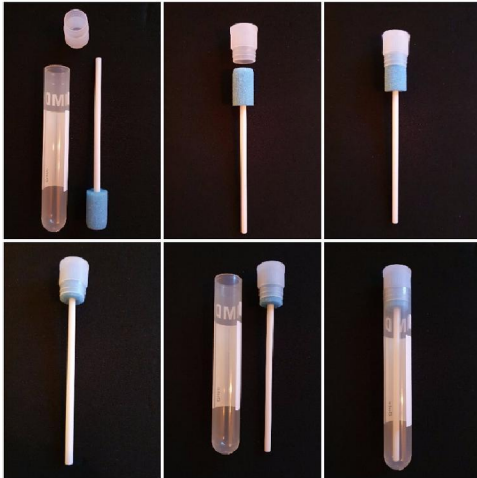
Oracol S10

Protocol
- No brushing teeth, eating, drinking other than water and no smoking at least half an hour before specimen collection
- Insert Oracol sponge between cheek and teeth and keep it there about two minutes moving back and forth (rolling it a bit)

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Specimen collection – Isohelix system

Protocol
- No brushing teeth, eating, drinking other than water and no smoking at least half an hour before specimen collection
- Drooling

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SARSLIVA – General aims:

(1) want to assess the sensitivity of self-sampling of saliva, in home situations, compared with NP and OP swabs in SARS-CoV-2 infected patients.

(2) assess whether saliva sampling is suitable for detecting low viral loads and tracing SARS-CoV-2 transmission, in asymptomatic and/or pre-symptomatic persons.

(3) Detect emerging mucosal IgA, IgM and IgG antibodies against SARS-CoV-2 (over time).

(4) Detect other respiratory viral pathogens (bacterial) in saliva, that may enhance of inhibit SARS-CoV-2 infection and symptoms.

SARSLIVA – Set-up

- Observational household cohort study, based on >75 index-cases with laboratory-confirmed COVID-19 and their household members.

- The index-patient is diagnosed to suffer from (laboratory-confirmed) COVID-19 by routine diagnostics using NP/OP swabs

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

SARSLIVA – sample collection

- 10 saliva samples per participant
- Isohelix collector
  - Saliva only
  - Saliva wit glycerol (1:1) for bacterial analyses (future presentation)
- Oracol sponge (children <5 years old)

|	1	3	5	7	10	14	21	28	35	42
Saliva	x	x	x	x	x	x	x	x	x	x

NP/OP

Serum
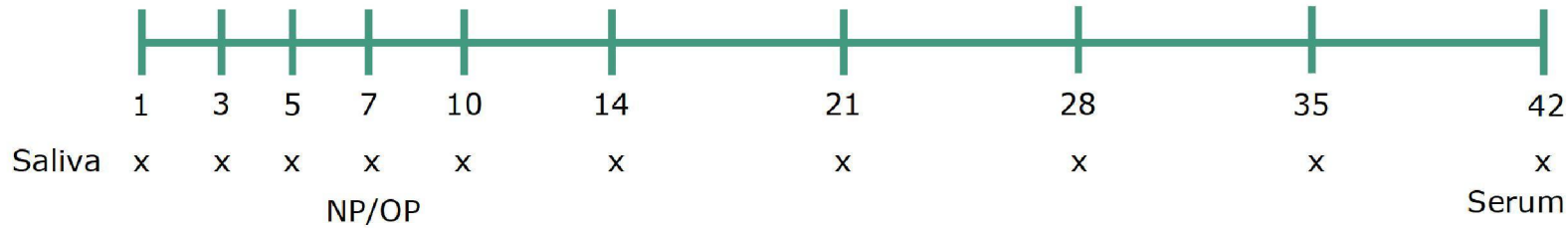
SARSLIVA - Results

Results - Families

- 88 families included
    - 2 "drop-outs"
    - **86** families for the complete duration of the study

- 330 family members included (including index cases)
    - 4 "drop-out"
    - **326** individuals for full duration of the study

- **3164** saliva specimens collected (out of 3260)
    - 27 specimens not enough material for RNA extraction (>200 ul)
    - Magnapure RNA extraction
    - Double target PCR for high sensitivity → Focus on E-target today (most sensitive)

Results – Household members

- 86 index "patients"
  - 82 also are PCR positive in saliva
    (NP/OP swap for inclusion is up to 3 days earlier)

- 221/326 individuals test positive in saliva
  - 200 are PCR positive at 2 or more timepoints
  - Most participants test positive at multiple timepoints
    - Median 6
    - Average 5,4
  - (Duration of shedding needs to be calculated &
    corrected for missing samples)
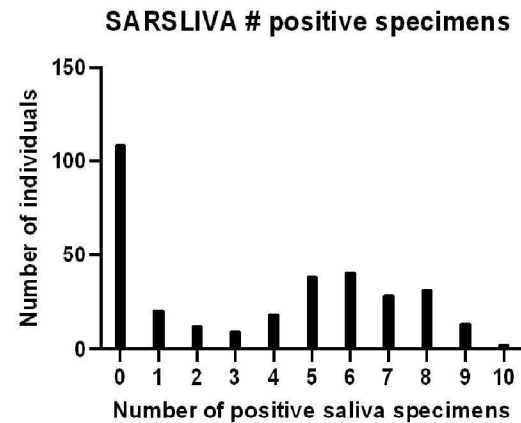
SARSLIVA # positive specimens

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Results – Household transmission

- In **68/86** households at least one of the household member got infected

Results – Household transmission

- In **68/86** households at least one of the household member got infected (very high attack rate)
    - In **46** families → transmission to multiple household members

Number of household members excl index (# households)				Number infected household members
2 (29)	3 (43)	4 (14)	5 (1)	
8	8	2	0	0
7	11	4	0	1
14	9	1	0	2
	15	4	0	3
		2	1	4
			0	5

- 104/139 contacts (non-index) that also got infected also test positive on t=1. (4/139 no data)

Results – Ongoing work/To Do (in random order)

- In 23 of 86 families included, **a child was the index patient**
  - 17/23 → transmission to at least one household member
  - Age categories need to be analyzed

- Comparison **OP/NP swap versus SALIVA**
  - Day 7 both an OP/NP and saliva sample has been analyzed
  - Need to be compared

- Analyze **Ct value (viral load) OP/NP swap** of index patient upon inclusion
  - Is there a correlation to level of household transmission

- **Serological** analyses (did we miss family members)
  - Wantai & Micro array
  - Any family members that are seropositive but always PCR negative?
  - Poor serological response in long term shedders?

Results – Ongoing work/To Do (in random order)

- **Antibody detection in Saliva**
  - Dynamics (in correlation to PCR detection, how long after infection is gone)
  - IgG/IgA

- **Sequence** analyses
  - Could there be a second introduction into the household
  - Especially relevant if one family member becomes PCR positive much later than other household members
  - Intra-host evolution for long-term shedders?
    (might be difficult due to higher Ct values at later timepoint)

- Transmission **dynamics** within family
  - Are there family members that were first negative and later positive
    (many were positive at t=1)
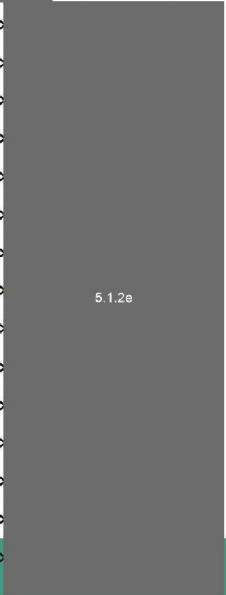 - Duration of shedding

Results – Ongoing work/To Do (in random order)

- **Co-infections**
    - Multiplex PCR (Streeklab/Spaarne)
    - Bacterial analyses (RIVM/UMCU, Rob, Willem, Krzysztof)

Acknowledgements

- RIVM-IDS
  - 5.1.2e & Ser Pools!
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