SARS-CoV-2 Viral Levels and Antibody Response in Mildly Symptomatic and Asymptomatic Participants

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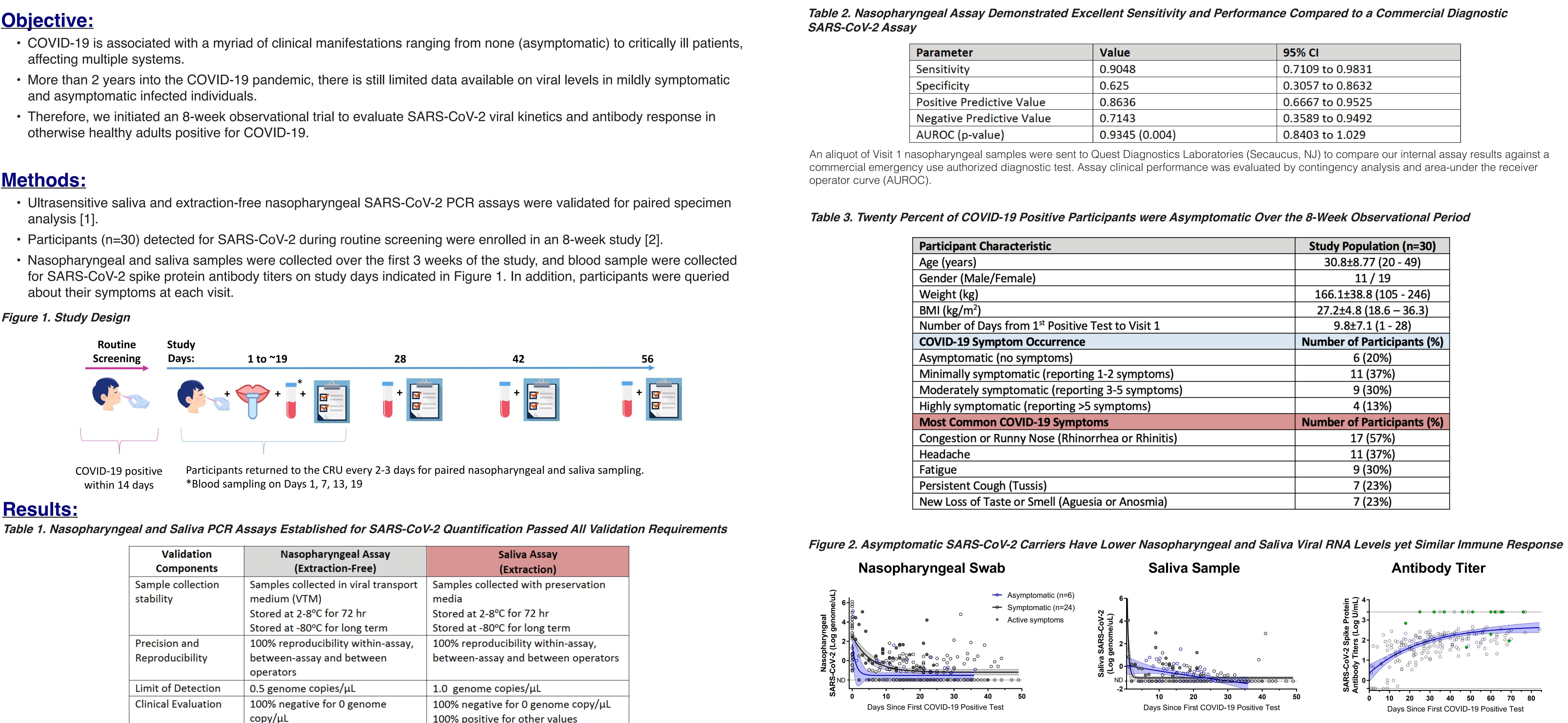Celerion, Lincoln, NE

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Objective:

- COVID-19 is associated with a myriad of clinical manifestations ranging from none (asymptomatic) to critically ill patients, affecting multiple systems.
- More than 2 years into the COVID-19 pandemic, there is still limited data available on viral levels in mildly symptomatic and asymptomatic infected individuals.
- Therefore, we initiated an 8-week observational trial to evaluate SARS-CoV-2 viral kinetics and antibody response in otherwise healthy adults positive for COVID-19.

Methods:

- Ultrasensitive saliva and extraction-free nasopharyngeal SARS-CoV-2 PCR assays were validated for paired specimen analysis [1].
- Participants (n=30) detected for SARS-CoV-2 during routine screening were enrolled in an 8-week study [2].
- Nasopharyngeal and saliva samples were collected over the first 3 weeks of the study, and blood sample were collected for SARS-CoV-2 spike protein antibody titers on study days indicated in Figure 1. In addition, participants were queried about their symptoms at each visit.

Figure 1. Study Design

Routine Screening → Study Days: 1 to ~19 | 28 | 42 | 56

COVID-19 positive within 14 days
Participants returned to the CRU every 2-3 days for paired nasopharyngeal and saliva sampling.
*Blood sampling on Days 1, 7, 13, 19

Results:

Table 1. Nasopharyngeal and Saliva PCR Assays Established for SARS-CoV-2 Quantification Passed All Validation Requirements
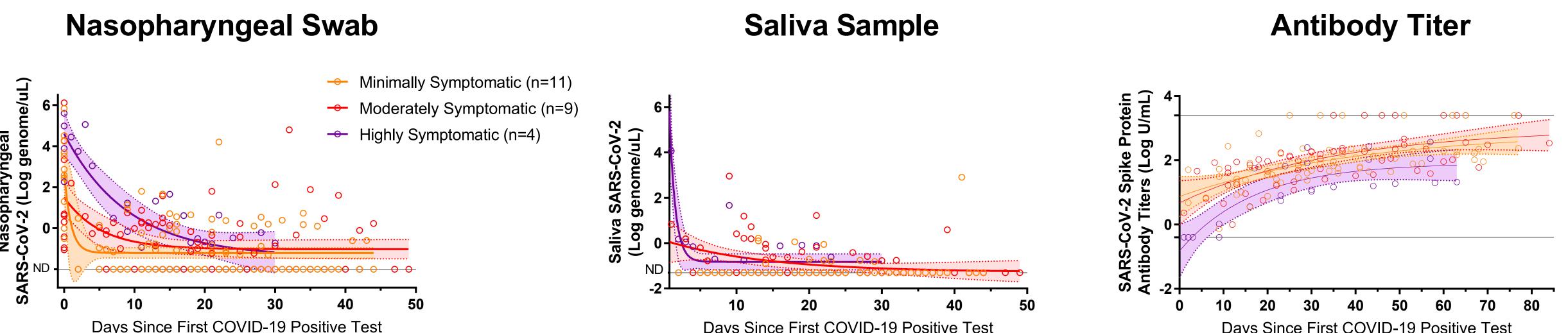
Validation Components	Nasopharyngeal Assay (Extraction-Free)	Saliva Assay (Extraction)
Sample collection stability	Samples collected in viral transport medium (VTM) Stored at 2-8°C for 72 hr Stored at -80°C for long term	Samples collected with preservation media Stored at 2-8°C for 72 hr Stored at -80°C for long term
Precision and Reproducibility	100% reproducibility within-assay, between-assay and between operators	100% reproducibility within-assay, between-assay and between operators
Limit of Detection	0.5 genome copies/µL	1.0 genome copies/µL
Clinical Evaluation	100% negative for 0 genome copy/µL 100% positive for other values	100% negative for 0 genome copy/µL 100% positive for other values
Freeze Thaw (FT) Stability	4 FT cycles established at -80°C	4 FT cycles established at -80°C
Short Term Stability	48 hours for N1 and RP reaction mix at 5°C	48 hours for N1 and RP reaction mix at 5°C

Nucleic acid extraction-free method uses less reagents and obtains results faster than a conventional procedure requiring nucleic acid extraction.

Table 2. Nasopharyngeal Assay Demonstrated Excellent Sensitivity and Performance Compared to a Commercial Diagnostic SARS-CoV-2 Assay

Parameter	Value	95% CI
Sensitivity	0.9048	0.7109 to 0.9831
Specificity	0.625	0.3057 to 0.8632
Positive Predictive Value	0.8636	0.6667 to 0.9525
Negative Predictive Value	0.7143	0.3589 to 0.9492
AUROC (p-value)	0.9345 (0.004)	0.8403 to 1.029

An aliquot of Visit 1 nasopharyngeal samples were sent to Quest Diagnostics Laboratories (Secaucus, NJ) to compare our internal assay results against a commercial emergency use authorized diagnostic test. Assay clinical performance was evaluated by contingency analysis and area-under the receiver operator curve (AUROC).

Table 3. Twenty Percent of COVID-19 Positive Participants were Asymptomatic Over the 8-Week Observational Period

Participant Characteristic	Study Population (n=30)
Age (years)	30.8±8.77 (20 - 49)
Gender (Male/Female)	11 / 19
Weight (kg)	166.1±38.8 (105 - 246)
BMI (kg/m²)	27.2±4.8 (18.6 – 36.3)
Number of Days from 1st Positive Test to Visit 1	9.8±7.1 (1 - 28)
COVID-19 Symptom Occurrence	**Number of Participants (%)**
Asymptomatic (no symptoms)	6 (20%)
Minimally symptomatic (reporting 1-2 symptoms)	11 (37%)
Moderately symptomatic (reporting 3-5 symptoms)	9 (30%)
Highly symptomatic (reporting >5 symptoms)	4 (13%)
Most Common COVID-19 Symptoms	**Number of Participants (%)**
Congestion or Runny Nose (Rhinorrhea or Rhinitis)	17 (57%)
Headache	11 (37%)
Fatigue	9 (30%)
Persistent Cough (Tussis)	7 (23%)
New Loss of Taste or Smell (Aguesia or Anosmia)	7 (23%)

Figure 2. Asymptomatic SARS-CoV-2 Carriers Have Lower Nasopharyngeal and Saliva Viral RNA Levels yet Similar Immune Response

Nasopharyngeal Swab | Saliva Sample | Antibody Titer

Nonlinear regression modeling revealed that asymptomatic viral levels are significantly lower than symptomatic counterparts, especially over the first week since testing positive (Nasopharyngeal: p=0.004; Saliva: p<0.0001). Not detected (ND) results hypothetically set at -2.0 and -1.3 Log genome/µL for nasopharyngeal and saliva, respectively. Participants with active symptoms during study visit are indicated by filled circles and vaccinated participants are indicated in green. Solid curves are best-fit models and shaded area represents 95% confidence intervals (CI).

Figure 3. Highly Symptomatic Participants Display Elevated Viral RNA and Reduced Immune Response

Nasopharyngeal Swab | Saliva Sample | Antibody Titer

Minimally Symptomatic (n=11)
Moderately Symptomatic (n=9)
Highly Symptomatic (n=4)

Nonlinear regression models of minimal, moderately and highly symptomatic participants. Solid curves are best-fit models and shaded area represents 95% CI.

Conclusion:

- Robust, sensitive analytical tools with low limits of detection are required to understand SARS-CoV-2 viral kinetics, especially in asymptomatic or mildly symptomatic COVID-19 participants.
- We established SARS-CoV-2 PCR assays able to detect 0.5 genome copies/µL in nasopharyngeal samples applying a nucleic acid extraction-free method, and saliva assay with limit of detection of 1 genome copies/µL.
- These ultrasensitive assays were applied in an observational trial, which followed asymptomatic and mildly symptomatic COVID-19 positive participants for 8 weeks.
- Asymptomatic participants displayed reduced nasopharyngeal (p=0.004) and saliva (p<0.0001) viral levels compared to symptomatic participants.
- In addition, we observed a symptom-dependent relationship with SARS-CoV-2 RNA levels in nasopharyngeal and saliva samples.
- Our findings suggest that reaching a viral load threshold may be a contributing factor to developing symptoms.

References:

1. Paglialunga S, Jaycox SH, Tavares T, Muruganandham A, Devaki B, Wattjes K et al. SARS-CoV-2 PCR Assay Validation – Nasopharyngeal vs Saliva Sample Collection. Celerion White Paper. 2022. www.celerion.com/wp-content/uploads/2022/05/SARS-CoV-2-Bioanalytical-PCR-Assay-Validation_White-Paper.pdf
2. Paglialunga S, Jaycox SH, Tavares T, Muruganandham A, Wattjes K, Bond R et al. SARS-CoV-2 Viral RNA Load is Associated with the Number of COVID-19 Symptoms Experienced in Mildly Symptomatic Adults: An Observational Study. Celerion White Paper. 2022. www.celerion.com/wp-content/uploads/2022/05/SARS-CoV-2-Viral-Kinetic-Observational-Study_White-Paper.pdf

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