SUPPLEMENTARY APPENDIX A: METHODOLOGY DETAILS

Description of Testing Center: The regional COVID-19 assessment center, termed "Brewer Park", is an assessment center for COVID-19 testing in Ottawa, ON for both adult and pediatric patients. Pediatric patients are seen in a separate section of the center. Tests were booked 0 to 72 hours in advance.

Study Recruitment Details: All children presenting to the Assessment Center were asked about their interest in research participation by the daytime Assessment Center staff (separate from the research team). The daytime staff were informed of our research study, and they identified patients who met indications for testing that were similar to our inclusion criteria. Interested participants who were identified by the daytime staff as meeting our inclusion criteria were flagged by the daytime staff, and subsequently approached by our research team. Our research team provided detailed study information to the patients and carried out a full assessment of their eligibility for inclusion. If patients were found to be eligible by the research team, they and/or their primary caregiver were consented for the research study. The research team was present onsite at almost all hours of the assessment center opening hours, however this varied depending on staffing numbers and availability. The research team provided information on the study and obtained informed consent from all participants.

List of Variants of Concern in Ontario during Study Period

January 2021: B.1.1.7 (Alpha)

February and March 2021: B.1.351 (Beta)

Table 1: Study Inclusion Criteria

Inclusion Criteria	***Ottawa Public Health Definition of High risk[1]**
1) Children aged 3-17	· A person who lived with someone who has tested positive, while that person was not self-isolating and infectious (includes congregate living settings where direct contact (<2 metres) is occurring in shared rooms/living spaces · A person who provided care for a person who has tested positive (bathing, toileting, dressing, feeding etc.) and/or had direct contact with infectious bodily fluids (e.g., coughed on or sneezed on) from up to 2 days (48 hours) before they became sick · A person who had other similar close (<2 metres) unprotected contact, for more than 15 minutes (the longer they were within 2 metres the higher the risk*), from up to 2 days (48 hours) before the person who tested positive was sick
AND AT LEAST ONE OF CRITERIA #2-4 BELOW:	
2) Child is identified as high risk or close contact to a confirmed positive case as defined by Ottawa Public Health*	
AND/OR	
3) Travelled outside Canada in the past 14 days	
AND/OR	

4) Exhibits signs or symptoms of COVID-19 viral illness a. **At least one of ("A" Symptom):** Fever, cough, shortness of breath, anosmia **<u>OR</u>** b. **Two or more of ("B" Symptom):** Congestion, sore throat, abdominal pain, vomiting, diarrhea, fatigue, loss of appetite, generalized muscle pain, headache	· Any patient in a healthcare setting in the same room when the person who tested positive was not on droplet and contact precautions or other patients in waiting room/common areas (i.e., < 2 metres from person for any duration of time) when the person who tested positive was not wearing a surgical/procedure mask · Passengers or crew (e.g. aircraft, train, bus, taxi) seated within 2 metres of the person who has tested positive (approximately two seats in all directions) and/or had close prolonged contact (more than 15 minutes) and/or direct contact with infectious bodily fluids, while the person who tested positive was not wearing a surgical/procedure mask · Any person who has travelled outside of Canada in the past 14 days

Table 2: Eligibility Criteria to quality for NP swab PCR test in Canada

Below lists the general eligbility criteria to qualify for a nasopharyngeal swab PCR test in Canada during the time of our study period.

January 2021	1. Anyone with any symptom consistent with COVID (though kids with minor single sx could watch & wait for 24 hrs) 2. Asymptomatic HRC and those receiving a COVID Alert App notification 3. Other Asx priority groups: Pre-procedure; Pre-placement into congregate settings; LTCH/RH residents, staff, contractors and visitors; First Nation/Indigenous/Metis/Inuit; International students completing quarantine 4. NOT ELIGIBLE – personal travel, peace of mind, knows a case but no exposure during period of communicability
February 2021	1. As above, PLUS: 2. UK VOC – encourage all returning travellers from UK and their contacts (even if Asx) to be tested
March 2021	3. As above, PLUS: 4. All kids in school/daycare with any symptoms required a test, as their household members were required to isolate pending the results 5. Confirmatory testing following + RAT 6. LTCH/RH testing was transitioning to RAT programs (several times per week)

	7. Added farm workers and educational workers (teachers, school bus drivers) to ASx priority group

Equations:

Eq. 1 $\boldsymbol{Kappa} = \frac{\boldsymbol{observed\ agreement - chance\ agreement}}{\boldsymbol{1 - chance\ agreement}}$

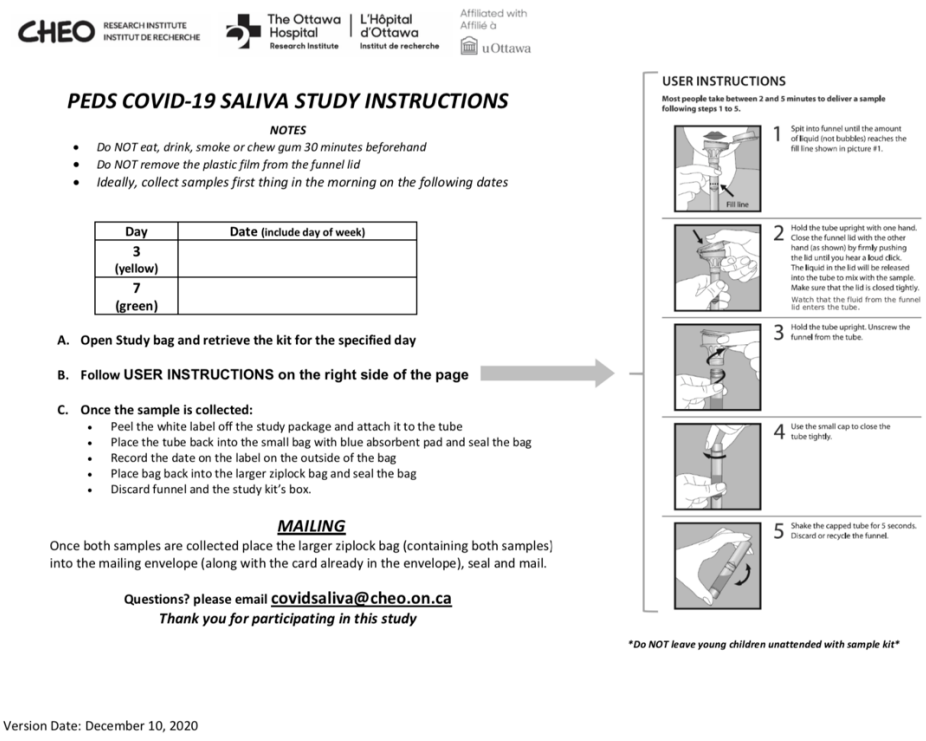
Eq. 2 PABAK $= \boldsymbol{2 * Observed\ agreement - 1}$

Eq. 3: $Prob\big((a)symptomatic\big) = \frac{S\ discordant\ and\ (a)symptomatic}{S\ (a)symptomatic}$

Appendix 1, as supplied by the authors. Appendix to: Hua N, Corsten M, Bello A, et al. Salivary testing for SARS-CoV-2 in the pediatric population: a diagnostic accuracy study. *CMAJ Open* 2022. DOI:10.9778/cmajo.20210279.

SUPPLEMENTARY APPENDIX B: Instruction Manuals for Families

Figure 1. Instruction Manual for self-collection saliva kit



CHEO RESEARCH INSTITUTE INSTITUT DE RECHERCHE | The Ottawa Hospital Research Institute | L'Hôpital d'Ottawa Institut de recherche | Affiliated with Affilié à uOttawa

PEDS COVID-19 SALIVA STUDY INSTRUCTIONS

NOTES

- *Do NOT eat, drink, smoke or chew gum 30 minutes beforehand*
- *Do NOT remove the plastic film from the funnel lid*
- *Ideally, collect samples first thing in the morning on the following dates*

Day	Date (include day of week)
3 (yellow)	
7 (green)	

A. Open Study bag and retrieve the kit for the specified day

B. Follow USER INSTRUCTIONS on the right side of the page

C. Once the sample is collected:

- Peel the white label off the study package and attach it to the tube
- Place the tube back into the small bag with blue absorbent pad and seal the bag
- Record the date on the label on the outside of the bag
- Place bag back into the larger ziplock bag and seal the bag
- Discard funnel and the study kit's box.

MAILING

Once both samples are collected place the larger ziplock bag (containing both samples) into the mailing envelope (along with the card already in the envelope), seal and mail.

Questions? please email covidsaliva@cheo.on.ca

Thank you for participating in this study

USER INSTRUCTIONS

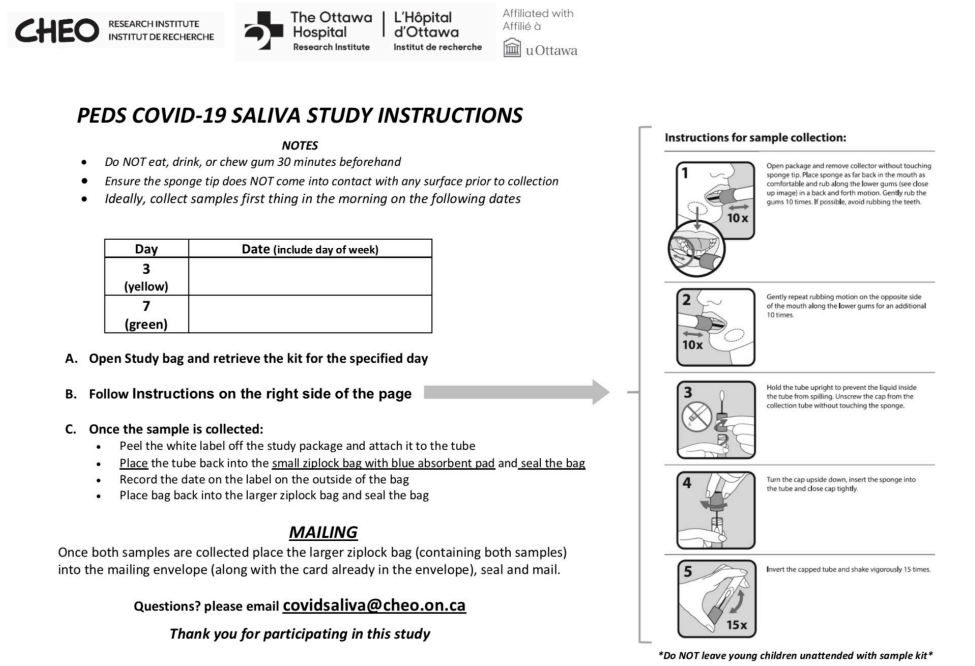
Most people take between 2 and 5 minutes to deliver a sample following steps 1 to 5.

1. Spit into funnel until the amount of liquid (not bubbles) reaches the fill line shown in picture #1.
2. Hold the tube upright with one hand. Close the funnel lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the sample. Make sure that the lid is closed tightly. Watch that the fluid from the funnel lid enters the tube.
3. Hold the tube upright. Unscrew the funnel from the tube.
4. Use the small cap to close the tube tightly.
5. Shake the capped tube for 5 seconds. Discard or recycle the funnel.

Do NOT leave young children unattended with sample kit

Version Date: December 10, 2020

Figure 2. Instruction Manual for self-collection sponge-stick saliva kit



PEDS COVID-19 SALIVA STUDY INSTRUCTIONS

NOTES

- *Do NOT eat, drink, or chew gum 30 minutes beforehand*
- *Ensure the sponge tip does NOT come into contact with any surface prior to collection*
- *Ideally, collect samples first thing in the morning on the following dates*

Day	Date (include day of week)
3 (yellow)	
7 (green)	

A. **Open Study bag and retrieve the kit for the specified day**

B. **Follow Instructions on the right side of the page**

C. **Once the sample is collected:**
- Peel the white label off the study package and attach it to the tube
- Place the tube back into the small ziplock bag with blue absorbent pad and seal the bag
- Record the date on the label on the outside of the bag
- Place bag back into the larger ziplock bag and seal the bag

MAILING

Once both samples are collected place the larger ziplock bag (containing both samples) into the mailing envelope (along with the card already in the envelope), seal and mail.

Questions? please email **covidsaliva@cheo.on.ca**

Thank you for participating in this study

Instructions for sample collection:

1 — Open package and remove collector without touching sponge tip. Place sponge as far back in the mouth as comfortable and rub along the lower gums (see close up image) in a back and forth motion. Gently rub the gums 10 times. If possible, avoid rubbing the teeth. 10x

2 — Gently repeat rubbing motion on the opposite side of the mouth along the lower gums for an additional 10 times. 10x

3 — Hold the tube upright to prevent the liquid inside the tube from spilling. Unscrew the cap from the collection tube without touching the sponge.

4 — Turn the cap upside down, insert the sponge into the tube and close cap tightly.

5 — Invert the capped tube and shake vigorously 15 times. 15x

Do NOT leave young children unattended with sample kit

SUPPLEMENTARY APPENDIX C:

Part 1: Introduction to longitudinal testing using a simulation

As discussed in the methods, we simulated a testing schedule that lasts for 30 days, with three scenarios where testing is performed every 2, 5, or 7 days, to assess and compare the hypothetical performance of repeat testing using NP- or saliva-based samples. Then we estimated the probability of detecting infection in an individual who was exposed any time before, during or after the testing schedule.

The sequential tests were assumed independent from one another. The simulations were based on modelling the kinetics of the viral load and the analytical sensitivity of the tests (based on NP and saliva samples). The viral load kinetics was modelled as a triangular function on the logarithm scale, parameterized on clinical studies.[2-4] The analytical sensitivity (i.e., sensitivity with respect to viral load present in the sample tested) was modelled with a logistic growth function parameterized on laboratory studies.[5-7] We assumed the analytical sensitivity of the NP-

based test to be 98% when the viral load is 10^{12} RNA copies per ml, 40% for 10^3 RNA copies/ml and a 99% specificity. For the saliva-based swab, we made the conservative assumption of a lower analytical sensitivity, with 85% when viral load is 10^{12} RNA copies per ml (the value of 85% was informed by the estimates reported in the result section of this longitudinal study) and 40% when it is $10^{3/0.85}$ RNA copies per ml. Note this simulation is based on a hypothetical population, not the pediatric cohort reported in this study.

Part 2: Model for longitudinal testing

To calculate the overall sensitivity of repeat testing with NP swabs or saliva samples, we modelled the time-dependent logarithmic concentration of SARS-CoV-2 with a triangular function and used a logistic curve to model the analytical sensitivity for both NP and saliva-based assays, making the conservative assumption that the saliva-based assay has a slightly lower analytical sensitivity.

A function, $S(v)$, represents the analytical sensitivity of the assay with respect to a given viral concentration v in the sample tested. The viral load (or concentration) in a patient that was infected a days ago is represented by the function $V(a)$. We model the sensitivity with respect to the time since infection by combining the analytical sensitivity $S(v)$ with the viral kinetics $V(a)$:

$$Se(a) = S(V(a))$$

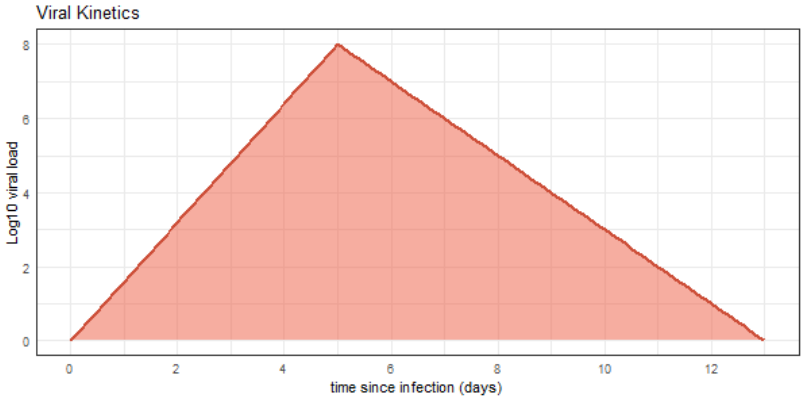
We model $log(V)$ as a triangular function with units as RNA copies per ml. The shape of this triangular function is informed by clinical studies.[8-10] The triangular function peaks on day 5 after infection at 10^8 RNA copies/ml and the limit of detection is reached 13 days after infection (Appendix C Figure 1).

There is a lack of experimental data that would allow to inform the precise shape of S. Hence, we model the analytical sensitivity of the test with a logistic function S such that $S(0) = 0.01$ (implicitly represents the test specificity). For the test based on the nasopharyngeal (NP) swab, we assume $S(10^{12}) = 0.98$ (i.e., the sensitivity of the test is 98% when the viral concentration is 10^{12} RNA copies/ml) and $S(10^{3}) = 0.4$. For the saliva-based test, we assume $S(10^{12}) = 0.85$ and $S\left(10^{3/0.85}\right) = 0.4$ (Appendix C Figure 1).

Then, we simulate a schedule of series independent tests (using the same type of assay within the schedule). For an individual infected at time x, and a given testing schedule where the tests occur at times t_i $(i = 1, \dots, n)$, we define the overall sensitivity of the testing schedule as:

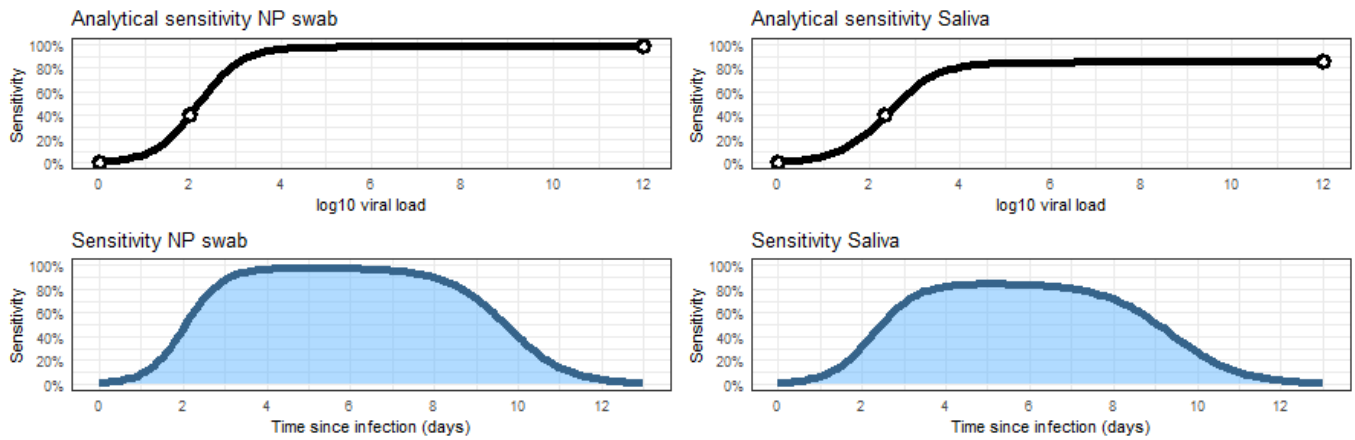
$$Se_{overall}(x) = 1 - \prod_{i=1}^{n}\left(1 - Se(\max(0, t_i - x)\right)$$

Appendix C Figure 1: Graphical representation of the model assumptions.



- *Viral Kinetics: Time since infection vs. Log10 Viral Load*

Appendix 1, as supplied by the authors. Appendix to: Hua N, Corsten M, Bello A, et al. Salivary testing for SARS-CoV-2 in the pediatric population: a diagnostic accuracy study. *CMAJ Open* 2022. DOI:10.9778/cmajo.20210279.



- Analytical Sensitivity model assumptions

Part 3: Interpretation of Longitudinal Testing Results

The following is a description on how to interpret the longitudinal testing result that is described in the main manuscript text, Figure 3.

We simulated a testing schedule that lasts for 30 days, with three scenarios where testing is performed every 2, 5, or 7 days. The x-axis represents the day that an individual is infected, with respect to the day of the first test of the testing schedule (t=0). The y-axis is sensitivity of the test. The vertical dashed lines indicate the day a test is performed.

Using the second graph 'Testing every 5 days' as an example, if an individual gets infected 19 days after the testing schedule starts, they have an 86% chance of testing positive from a saliva sample some time during the testing schedule. This patient will most likely have a negative test on day T=20, because their viral load is not high enough just one day after infection. However, on testing day T=25, their test will likely be positive at that time, because this is 6 days after infection and viral load is likely close to its peak value. Moreover, there is still another chance that on testing day T=30 their test will be (again) positive because infected patients may keep on shedding for a while. Hence, this 86% sensitivity for a patient infected on day T=19 is calculated

by combining all the chances that all the next tests in the testing schedule will be positive: extremely small chance for T=20, large chance for T=25, fair chance for T=30.

As seen in the graphs, the sensitivity drops towards zero at the end of the testing schedule. For a patient infected on day T=29, there is just one test (chance) left on day T=30 to identify their infection, which is very unlikely (just one day of infection is likely not enough to be detected by a test).

Based on this simulation, a patient that is tested more frequently has a higher chance to be picked up on salivary testing, thereby increasing the sensitivity of the test. This highlights the importance of repetitive testing and highlights the feasibility of using saliva testing in COVID-19 surveillance programs.

Appendix 1, as supplied by the authors. Appendix to: Hua N, Corsten M, Bello A, et al. Salivary testing for SARS-CoV-2 in the pediatric population: a diagnostic accuracy study. *CMAJ Open* 2022. DOI:10.9778/cmajo.20210279.

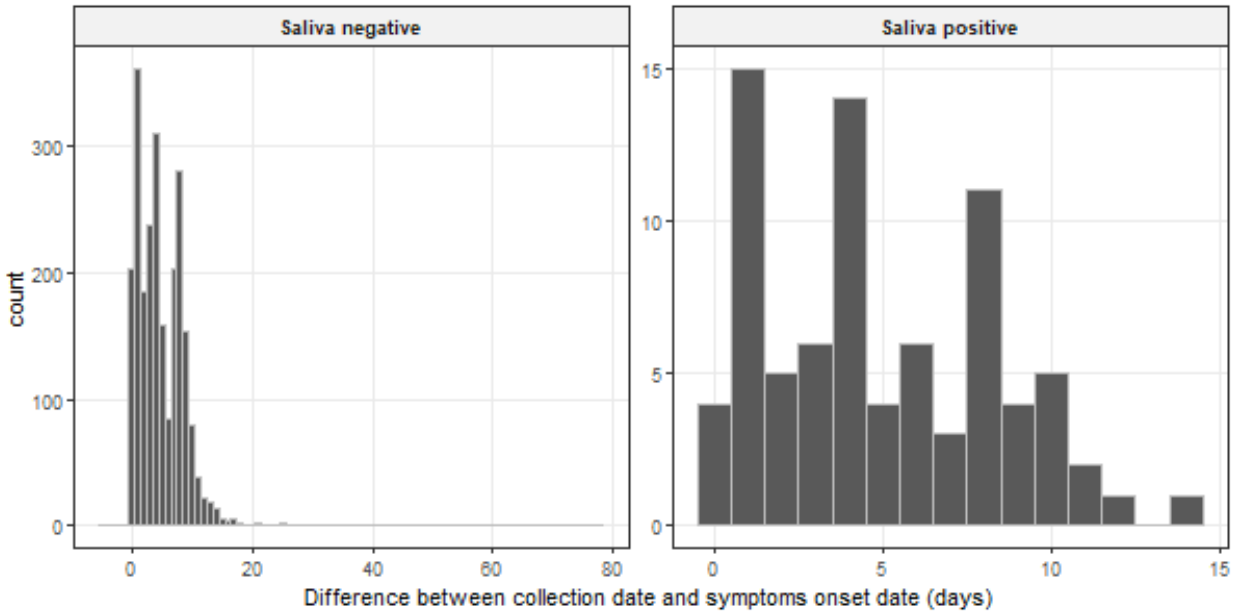
SUPPLEMENTARY APPENDIX D: Additional Results

Table 1: Stratification of participants' concordant status by presence or absence of symptoms.

	Concordant		**Discordant**		
	Positive	**Negative**	**NP swab positive**	**Saliva-based assay positive**	**Total**
Symptomatic:	33	994	1	4	1032
Asymptomatic:	11	526	7	4	548
Total	44	1520	8	8	

Figure 1: Distribution of the difference of time between saliva sample collection date and reported symptom onset date; (A) Distribution by absolute count; (B) Distribution by density

(A)



(B)

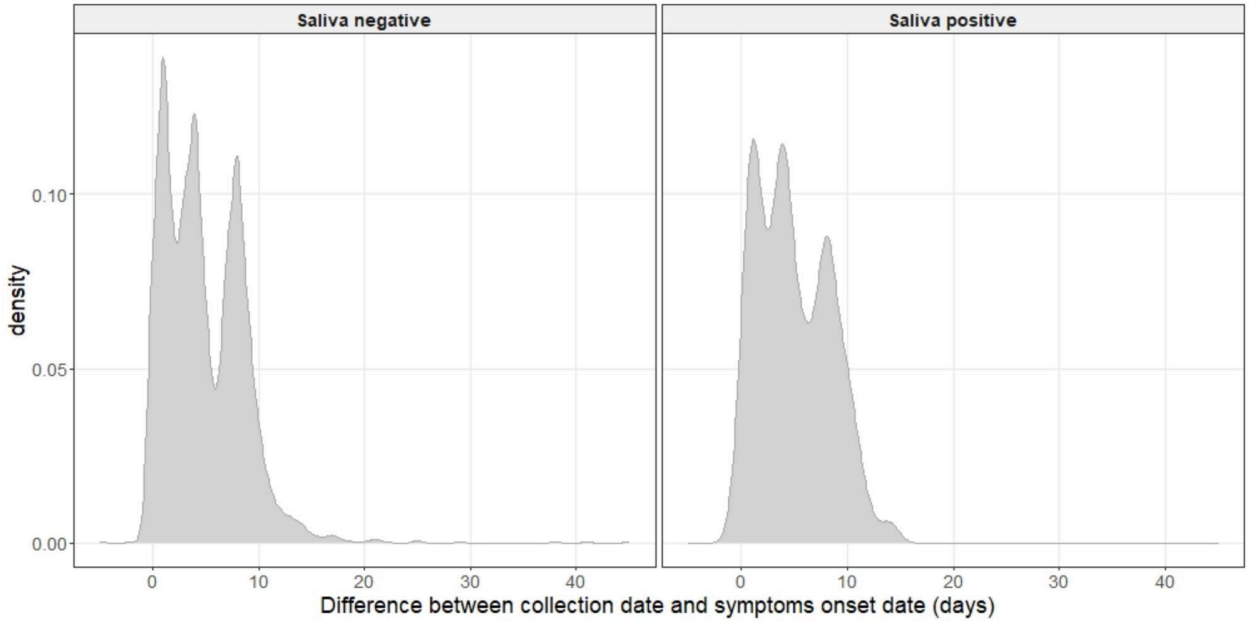
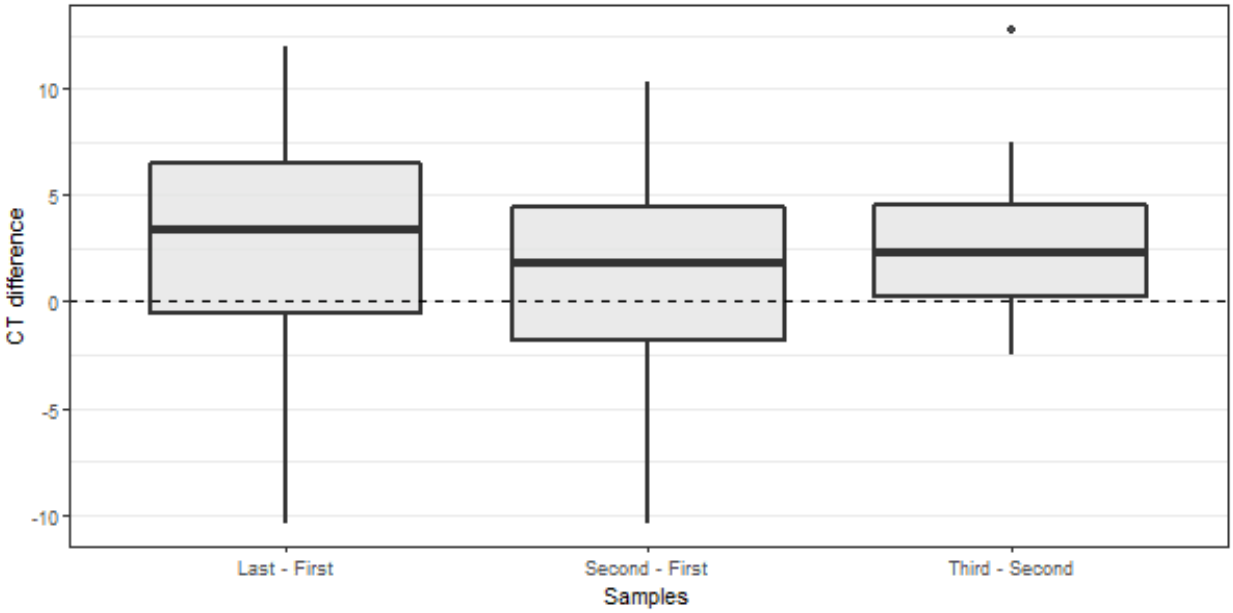


Figure 2: Difference of CT values for saliva samples.



"Last - First" is the calculation between the first and last sample (irrespective of the total number of positive samples, as long as there are at least two).

"Second - First" is the calculation between the second sample that was positive and the first that was positive.

"Third - Second" is the CT difference between the third and second samples that were positive (only for patients that had three positive results for their saliva-based samples).

References

1. COVID-19 high risk contacts. Ottawa: Ottawa Public Health; 2021. Available: https://www.ottawapublichealth.ca/en/shared-content/covid-19-high-risk-contacts.aspx (accessed 2021 Sept. 5). </eref>
2. Weiss A, Jellingsø M, Morten OAS. Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: a systematic review and meta-analysis. *EBioMedicine* 2020;58:102916.
3. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;581:465-9. doi: 10.1038/s41586-020-2196-x.
4. Cevik M, Tate M, Lloyd O, et al. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* 2021;2:e13-e22.
5. Vogels CB, Brito A, Wyllie A, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. *Nat Microbiol* 2020;5:1299-305.
6. Fung B, Gopez A, Servellita V, et al. Direct comparison of SARS-CoV-2 analytical limits of detection across seven molecular assays. *J Clin Microbiol* 2020;58:e01535-20.
7. Smith E, Zhen W, Manji R, et al. Analytical and clinical comparison of three nucleic acid amplification tests for SARS-CoV-2 detection. *J Clin Microbiol* 2020;58.
8. Fougère Y, Schwob J, Miauton A, et al. Performance of RT-PCR on saliva specimens compared with nasopharyngeal swabs for the detection of SARS-CoV-2 in children. *Pediatr Infect Dis J* 2021;40:e300-4.
9. Li L, Shim T, Zapanta P. Optimization of COVID-19 testing accuracy with nasal anatomy education. *Am J Otolaryngol* 2021;42:102777.
10. Caulley L, Corsten M, Eapen L, et al. Salivary detection of COVID-19. *Ann Intern Med* 2021;174:131-3.

Appendix 1, as supplied by the authors. Appendix to: Hua N, Corsten M, Bello A, et al. Salivary testing for SARS-CoV-2 in the pediatric population: a diagnostic accuracy study. *CMAJ Open* 2022. DOI:10.9778/cmajo.20210279.