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RESEARCH ARTICLE

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Food for thought: Eating before saliva collection and interference with SARS-CoV-2 detection

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Abstract

Saliva is a promising specimen for the detection of viruses that cause upper respiratory infections including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to its cost-effectiveness and noninvasive collection. However, together with intrinsic enzymes and oral microbiota, children's unique dietary habits may introduce substances that interfere with diagnostic testing. To determine whether children's dietary choices impact SARS-CoV-2 molecular detection in saliva, we performed a diagnostic study that simulates testing of real-life specimens provided from healthy children ($n = 5$) who self-collected saliva at home before and at 0, 20, and 60 min after eating 20 foods they selected. Each of 72 specimens was split into two volumes and spiked with SARS-CoV-2-negative or SARS-CoV-2-positive clinical standards before side-by-side testing by reverse-transcription polymerase chain reaction matrix-assisted laser desorption ionization time-of-flight (RT-PCR/MALDI-TOF) assay. Detection of internal extraction control and SARS-CoV-2 nucleic acids was reduced in replicates of saliva collected at 0 min after eating 11 of 20 foods. Interference resolved at 20 and 60 min after eating all foods except hot dogs in one participant. This represented a significant improvement in the detection of nucleic acids compared to saliva collected at 0 min after eating ($p = 0.0005$). We demonstrate successful detection of viral nucleic acids in saliva self-collected by children before and after eating a variety of foods. Fasting is not required before saliva collection for SARS-CoV-2 testing by RT-PCR/MALDI-TOF, but waiting for 20 min after eating is sufficient for accurate testing. These findings should be considered for SARS-CoV-2 testing and broader viral diagnostics in saliva specimens.

KEYWORDS

interference, MALDI-TOF, RT-PCR, saliva, SARS-CoV-2

1 | INTRODUCTION

Robust diagnostics are vital for testing respiratory viral infections including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to prevent transmission from infected children. Although many nucleic acid amplification platforms for SARS-CoV-2 detection have been authorized by the United States Food and Drug Administration (US FDA) for testing nasopharyngeal (NP) specimens, collection involves invasive techniques and close contact with infected individuals.[1] Saliva is an attractive alternative since the collection is less invasive, less uncomfortable, and saliva can be self-collected.

We[2] and others[3–6] have demonstrated the diagnostic utility of saliva for SARS-CoV-2 testing, and several have demonstrated comparable sensitivities for SARS-CoV-2 detection in saliva versus NP specimens,[5,7] but few have assessed performance in children[4,5] whose saliva can be impacted by dietary and oral hygiene behaviors distinct from adults. With routine self-collection, robust detection of viruses is vulnerable to various inhibitors found in saliva secondary to dental care, microbiota, native salivary environment, and foods in everyday diets.[8–10]

To date, few studies assess the capacity of substances to interfere with SARS-CoV-2 molecular diagnostics in routinely collected saliva specimens that comprise scalable surveillance programs across the country.[11–14] In addition, in attempts to avoid inhibitors, programs utilize inconsistent protocols for collecting saliva from subjects that range from fasting to collecting upon waking or to coughing before generating clinical specimens (reviewed in [14]). Together, these factors highlight the need to evaluate the impacts of potential dietary inhibitors found in specimens self-collected in routine settings.

To evaluate whether dietary choices can impact SARS-CoV-2 detection in saliva, we performed for the first time a proof-of-point methodologic study to simulate testing of real-life specimens provided from healthy children who self-collected saliva at home before and after eating foods they selected. Our main objective was to investigate the inhibitory effects of each child's favorite child food types and its potential impact on polymerase chain reaction (PCR) inhibition only from a methodological standpoint. Although the small number of subjects included in this study precludes drawing conclusions at a larger population scale, the diverse food components tested do allow timely inferences to be made to achieve optimal analytical success at the time of testing. Our results showed successful detection of SARS-CoV-2 viral nucleic acids in saliva self-collected by children before and after eating a variety of foods. These findings have relevant implications in terms of using saliva specimens for detecting other viral agents in the future.

2 | METHODS

2.1 | Ethics statement

This study was reviewed and approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai (HS#21-00670). Consent was obtained from at least one parent of each child participant.

2.2 | Foods evaluated for interference with target detection

A panel of 20 "favorite" foods was identified by the participants who also collaborated in the study design. Detailed information on these foods is provided in Table S1. Participants' renderings of select foods are depicted in Figures 1A and S1–4.

2.3 | Saliva collection

Saliva specimens were provided by five healthy children aged between 5 and 9 years. Children generated at-home, self-collected specimens in 15 ml sterile conical vials for SARS-CoV-2 molecular testing. Participants provided saliva once immediately upon waking up before any dental care, and then at 0, 20, and 60 min after eating each food. Parents annotated the food, timepoint, and date of specimen collection on each tube with a marker. Specimens were refrigerated in biohazard bags for less than 72 h after collection before transfer to the Mount Sinai Health System Clinical Laboratory, which is certified under Clinical Laboratory Improvement Amendments of 1988, 42 U.S.C. §263a and meets requirements to perform high-complexity tests. Of note, at each timepoint, each participant provided 1–2.5 ml of saliva specimen.

2.4 | SARS-CoV-2 molecular testing

Upon receipt, specimen volumes were divided in half to undergo artificial spiking with in-house standards before diagnostic testing using the Agena MassARRAY® SARS-CoV-2 Panel and MassARRAY® System (Agena; CPM384) platform as previously described.[2] This method has been validated for clinical testing and has received Emergency Use Authorization (EUA) by the US FDA. Each saliva specimen collected was split into two equal volumes for spiking with SARS-CoV-2-positive or SARS-CoV-2-negative standards. One volume was spiked with a quantitated standard of pooled SARS-CoV-2-positive NP specimens.[2] Specifically, for each replicate, 18.75 µl of SARS-CoV-2-positive NP specimen was added to 281.25 µl of saliva specimen. Depending on the volume of saliva provided by participants for each timepoint, this generated 1–3 technical replicates of saliva at 300 µl each containing 125,000 genome copies/ml of SARS-CoV-2. The second volume of saliva specimen was spiked with pooled SARS-CoV-2-negative NP matrix standard; similarly, for each replicate, 18.75 µl of SARS-CoV-2-negative NP specimen was added to 281.25 µl of saliva specimen; this generated 1–3 replicates of saliva at 300 µl each containing no SARS-CoV-2. Specimen replicates were processed and run side-by-side as previously described.[2] Per the diagnostic protocol under US FDA EUA, commercial MS2 phage

FIGURE 1 Foods selected by participants to test for the interference of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostic testing in saliva. (A) Participants' rendering of select categories of foods tested for impact on SARS-CoV-2 detection in saliva. (B) Participants' rendition of the process of collecting saliva, artificially spiking with (or without) SARS-CoV-2, RNA extraction, and reverse-transcription polymerase chain reaction matrix-assisted laser desorption ionization time-of-flight diagnostic testing. Participants' renderings were provided by the following authors: D. E. P.-P., P. A. P.-P., A. L. R., J. D., D. D., S. B., and L. B.

nucleic acid reagent was used as an internal extraction control (IC; concentration proprietary and unavailable).[15]

Data acquired by the MassARRAY® Analyzer were processed with the MassARRAY® Typer and SARS-CoV-2 Report software. The assay detects five viral targets: three in the *N* gene (N1, N2, N3) and two in the *ORF1ab* gene (ORF1A, ORF1AB). Assay and target results were interpreted as defined by the manufacturer's instructions for use under FDA EUA.[15] Briefly, if the commercial MS2 phage IC was detected, results were interpreted as negative if <2 targets were detected. If no IC and no targets were detected, the result was invalid. However, if ≥2 SARS-CoV-2 targets were detected, the result was positive, regardless of the IC detection result. Detailed diagnostic results for all replicates are described in Table S2. Participants' depictions of methods are portrayed in Figures S5 and S6. Mass-spectrometry spectra representative of possible outcomes observed in this study are depicted in Figures S7–11.

2.5 | Statistical analyses and display items

To compare detection frequency results at different timepoints of all saliva specimens tested, normality was assessed by D'Agostino and Pearson test and Wilcoxon matched-pairs signed-rank test was performed (GraphPad Prism 9.0.2).

3 | RESULTS

3.1 | Interference of extraction control detection in saliva

Pediatric participants independently selected 20 of their favorite foods and provided saliva for reverse-transcription PCR matrix-assisted laser desorption ionization time-of-flight (RT-PCR/MALDI-TOF) detection of artificially-added viral and IC nucleic acids (Figure 1). Altogether, 72 different saliva specimens were collected before and after eating. All specimens were divided into two volumes to undergo side-by-side testing after spiking with in-house SARS-CoV-2-negative or SARS-CoV-2-positive standards. In total, 404 replicates were tested.

We assessed the successful extraction and detection of ICs in specimens that were spiked with SARS-CoV-2-negative standard (Table 1). First, morning saliva collected immediately upon waking was collected from each participant. Morning saliva from four participants yielded successful detection of IC and a negative result for SARS-CoV-2 in 100% of replicates. In contrast, IC was detected in 0/3 replicates (0%) of participant #1 morning saliva, suggesting the presence of substances that inhibit extraction or detection of IC. Morning saliva from a second independent collection for this participant yielded IC detection in 2/3 replicates (67%), which still reflects a level of interference.

When we assessed interference in saliva collected after eating, we found the greatest interference with IC detection occurred in specimens collected immediately after eating (0 min) (Table 1). In saliva collected immediately after eating 12 foods, IC was detected in 0/3 (0%) to 2/3 (67%) replicates. However, for 11 of the 12 foods, IC was detected in 100% of replicates at 20 and 60 min after eating, representing a significant improvement from the 0 min timepoint ($p = 0.0005$). Interestingly, saliva collected after eating a hot dog was the exception and interfered with IC detection up to 60 min after consumption in participant #1. Notably, participant #3 also consumed hot dog, but IC was detected in all replicates from 0 to 60 min after eating.

3.2 | Interference of SARS-CoV-2 nucleic acid detection in saliva

We next assessed the effect of eating before saliva collection on SARS-CoV-2 detection in spiked samples. To do this, we spiked specimens with SARS-CoV-2-positive standard to generate replicates of specimens each containing 125 000 genome copies/ml. This reflects a concentration that is ~100-fold greater than the limit of detection of the assay, but is <10-fold greater than the diagnostic target (ORF1AB) with the lowest analytical sensitivity in saliva clinical matrix.[2] This was done to exclude the impacts of distinct target analytic performance when challenged by various interfering substances.

SARS-CoV-2 detection was uniformly successful in SARS-CoV-2-spiked morning saliva specimens from four participants; however, SARS-CoV-2 was not detected in spiked saliva from participant #1 (Table 2). Together with the failure to detect IC in morning saliva from the same participant, these findings suggest that morning saliva may have substances that inhibit extraction, amplification, and/or detection of nucleic acids. In fact, when participant #1 morning saliva collected at a second independent collection was spiked with SARS-CoV-2, viral nucleic acids were detected in 0/3 replicates (Table S2).

Similar to saliva spiked with SARS-CoV-2-negative standard, the greatest interference with detection occurred in specimens collected immediately after eating (Table 2). Specifically, SARS-CoV-2 was detected in 0%–50% of all replicates from SARS-CoV-2-spiked saliva collected immediately after eating 11 foods. These same foods were all associated with inhibition of detection of IC nucleic acids in paired specimens spiked with SARS-CoV-2-negative standard. Interestingly, for participant #3, contrary to detection of IC in SARS-CoV-2-negative saliva collected immediately after eating a hot dog, viral nucleic acids were detected in 0/3 replicates (0%) of paired saliva spiked with SARS-CoV-2. Successful detection of SARS-CoV-2 was achieved in saliva collected at 20–60 min after eating almost all (19/20) foods, which represents a significant improvement in detection compared to the 0 min timepoint ($p = 0.0010$). Once again, the exception was for participant #1 saliva collected after eating a hot dog, which interfered with SARS-CoV-2 detection up to 1 h later.

TABLE 1 Detection of internal extraction control in children's saliva before and after eating

Participant	Morning[b]	After eating[a] Food	0 min	20 min	60 min
1	0/3 (0%)	–	–	–	–
	–	Hot dog	0/3 (0%)	2/3 (66.7%)	2/3 (66.7%)
	–	Ice cream	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Cookies	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Pizza	0/2 (0%)	3/3 (100%)	3/3 (100%)
	–	Pretzels	0/3 (0%)	3/3 (100%)	3/3 (100%)
2	3/3 (100%)	–	–	–	–
	–	Apple	2/2 (100%)	2/2 (100%)	3/3 (100%)
	–	Banana	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Chocolate	2/3 (66.7%)	3/3 (100%)	3/3 (100%)
	–	Clementine	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Hamburger	3/3 (100%)	2/2 (100%)	2/2 (100%)
	–	Chicken nuggets	0/2 (0%)	3/3 (100%)	3/3 (100%)
	–	Pasta	1/2 (50.0%)	3/3 (100%)	3/3 (100%)
	–	Popcorn	2/2 (100%)	3/3 (100%)	2/2 (100%)
	–	Waffle	0/3 (0%)	3/3 (100%)	2/2 (100%)
3	3/3 (100%)	–	–	–	–
	–	Eggs and bacon	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Macaroni and cheese	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Peanut butter and jelly	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Potato chips	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Hot dog	3/3 (100%)	3/3 (100%)	3/3 (100%)
4	1/1 (100%)	–	–	–	–
	–	French fries	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Pancake	3/3 (100%)	3/3 (100%)	3/3 (100%)
5	3/3 (100%)	–	–	–	–
	–	French fries	2/2 (100%)	2/2 (100%)	3/3 (100%)
Overall	10/13 (76.9%)	–	30/60 (50.0%)	62/63 (98.4%)	62/63 (98.4%)

Abbreviations: NP, nasopharyngeal; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Fraction of saliva replicates collected after eating that is spiked with SARS-CoV-2-negative NP matrix and yield detectable internal extraction control. Fraction is also depicted as a percentage.

[b]Fraction of saliva replicates collected in the morning that is spiked with SARS-CoV-2-negative NP matrix and yield detectable internal extraction control. Fraction is also depicted as a percentage.

4 | DISCUSSION

As children return to in-person schooling, large-scale SARS-CoV-2 screening and surveillance efforts have utilized saliva for its cost-effective, noninvasive, and safe characteristics in testing children and adults.[16–19] However, to effectively execute these initiatives and to exploit saliva for testing of other viruses, a better assessment of the diagnostic performance of saliva in children is required. In particular, the requirement to fast or avoid eating may be difficult for parents and children because of constraints on mealtime scheduling and the unpredictability of children's dietary habits.

To address this, the children who participated in our study collected saliva upon waking, and then, at set intervals, after eating their favorite foods. Although we highlight as a limitation of our study the

TABLE 2 Detection of SARS-CoV-2 in children's saliva before and after eating

Participant	Morning[b]	After eating[a] Food	0 min	20 min	60 min
1	0/3 (0%)	–	–	–	–
	–	Hot dog	0/3 (0%)	2/3 (66.7%)	2/3 (66.7%)
	–	Ice cream	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Cookies	1/3 (33.3%)	3/3 (100%)	3/3 (100%)
	–	Pizza	1/2 (50.0%)	3/3 (100%)	3/3 (100%)
	–	Pretzels	0/3 (0%)	3/3 (100%)	3/3 (100%)
2	3/3 (100%)	–	–	–	–
	–	Apple	2/2 (100%)	2/2 (100%)	3/3 (100%)
	–	Banana	1/3 (33.3%)	3/3 (100%)	3/3 (100%)
	–	Chocolate	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Clementine	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Hamburger	3/3 (100%)	2/2 (100%)	2/2 (100%)
	–	Chicken nuggets	1/2 (50.0%)	3/3 (100%)	3/3 (100%)
	–	Pasta	1/2 (50.0%)	3/3 (100%)	3/3 (100%)
	–	Popcorn	2/2 (100%)	3/3 (100%)	2/2 (100%)
	–	Waffle	0/3 (0%)	3/3 (100%)	2/2 (100%)
3	3/3 (100%)	–	–	–	–
	–	Eggs and bacon	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Macaroni and cheese	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Peanut butter and jelly	3/3 (100%)	3/3 (100%)	3/3 (100%)
	–	Potato chips	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Hot dog	0/3 (0%)	3/3 (100%)	3/3 (100%)
4	1/1 (100%)	–	–	–	–
	–	French fries	0/3 (0%)	3/3 (100%)	3/3 (100%)
	–	Pancake	3/3 (100%)	3/3 (100%)	3/3 (100%)
5	3/3 (100%)	–	–	–	–
	–	French fries	2/2 (100%)	2/2 (100%)	3/3 (100%)
Overall	10/13 (76.9%)	–	32/60 (53.3%)	62/63 (98.4%)	62/63 (98.4%)

Abbreviations: NP, nasopharyngeal; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Fraction of saliva replicates collected after eating that is spiked with SARS-CoV-2-positive NP matrix and yield detectable SARS-CoV-2. Fraction is also depicted as a percentage.

[b]Fraction of saliva replicates collected in the morning that is spiked with SARS-CoV-2-positive NP matrix and yield detectable SARS-CoV-2. Fraction is also depicted as a percentage.

small number of participants, we did assess a broad range of food types consumed by children, with the consistent finding that, by 20 min after eating most foods, there was minimal or no interference with extraction, amplification, and detection of IC and SARS-CoV-2. Importantly, specific foods (e.g., hot dogs) and components in saliva collected upon waking up may interfere with diagnostics, which warrants further study on other RT-PCR-based and novel (e.g., RT loop-mediated isothermal amplification, CRISPR/Cas-based) diagnostic platforms. In addition, relative to the limit of detection of the assay, this study utilized a high viral copy number to exclude the effects of distinct targets with low analytic sensitivity (e.g., ORF1AB). This may underestimate the inhibitory potential of each of the foods children selected. Therefore, larger, follow-up studies are warranted to evaluate the effects of such inhibitory substances on the analytic sensitivities of these molecular assays and of their individual component targets.

Although limited in size, this study benefits from the fact that it simulates routine self-collection from children and assesses the

impacts of diverse substances likely encountered in ongoing, real-life SARS-CoV-2 surveillance programs across communities worldwide. Indeed, participants selected and tested an array of foods that are both diverse in molecular composition (e.g., fat, carbohydrates, etc.) and are commonly found in children's diets. Furthermore, these findings have the potential to inform ongoing and developing SARS-CoV-2 surveillance programs as well as broader molecular diagnostics designed to target various respiratory pathogens in saliva including adenovirus, respiratory syncytial, and influenza viruses.[8–10,20]

Despite its advantages, saliva, like other biological samples, is prone to matrix-specific factors that influence diagnostics including intrinsic degrading enzymes, changes in salivary flow over time (e.g., circadian rhythms), or oral microbiome composition.[8–10,20] Moreover, various PCR inhibitors have been described in milk, vegetables, and foods high in protein and fat which interfere with robust diagnostic detection of pathogen nucleic acids.[8,9] Together with the unique dietary habits of children, these factors pose challenges to molecular microbiology diagnostics and are essential to consider as we learn to exploit saliva for capturing infections with upper respiratory pathogens.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Mary C. Boyle and Mariawy Riollano-Cruz recruited and consented parents and subjects to provide specimens for this study. Daniel E. Paniz-Perez, Paul A. Paniz-Perez, Aryan L. Rishi, Jacob Dubinsky, Dylan Dubinsky, Sophie Baine, and Lily Baine participated in the design and implementation of the research. Matthew M. Hernandez, Radhika Banu, Paras Shrestha, Liyong Cao, Feng Chen, and Alberto E. Paniz-Mondolfi accessioned subjects' specimens and performed diagnostic assays. Matthew M. Hernandez, Radhika Banu, Huanzhi Shi, Emilia Mia Sordillo, and Alberto E. Paniz-Mondolfi analyzed, interpreted, or discussed data. Suzanne Arinsburg, Ian Baine, and Carlos Cordon-Cardo contributed to the interpretation of the results. Matthew M. Hernandez, Emilia Mia Sordillo, and Alberto E. Paniz-Mondolfi wrote the manuscript. Matthew M. Hernandez and Alberto E. Paniz-Mondolfi conceived the study. Emilia Mia Sordillo and Alberto E. Paniz-Mondolfi supervised the study. All authors discussed the results and commented on the manuscript. Matthew M. Hernandez and Alberto E. Paniz-Mondolfi are the guarantors of this study and, as such, had full access to all of the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting information of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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