



Letter

Comparing methods to collect saliva from children to analyze cytokines related to allergic inflammation

Human saliva is a complex fluid rich in immunologic components, which reflects real-time systemic concentrations. Advances in biotechnology have enabled us to measure minute concentrations of immunologic components, such as cytokines, in saliva samples with precision and accuracy.¹ There are few data evaluating how the collection method may influence the results in addition to other methodologic challenges, resulting in lack of consensus on a universally accepted saliva collection technique,² especially in children. Specifically, the effect of saliva collection methods on detection and analysis of salivary concentrations of allergic inflammatory cytokines, such as the interleukin (IL) 4, IL-5, IL-13, eotaxin 3 (Eo3), and thymic stromal lymphopoietin (TSLP), that mediate allergic inflammation³ has not been reported. These cytokines play an important role in the development of allergic inflammatory states in increasingly prevalent conditions, such as allergic rhinitis, food allergy, asthma, atopic dermatitis, and eosinophilic esophagitis.⁴

The overall goal of this study was to compare 2 methods of saliva collection in the detection and analysis of salivary concentrations of IL-4, IL-5, IL-13, Eo3, and TSLP in children. Our study was approved by the Baylor Institutional Review Board, and informed consent was obtained from the parent and assent, where appropriate, from the child.

We studied 20 children (median age, 14 years; interquartile range, 9–17 years), 14 (64%) of whom were female and 13 (59%) of whom were white. Three children (15%) did not report any allergies, and the remaining 17 children (85%) reported 1 or more of the following commonly prevalent allergic conditions: atopic dermatitis ($n = 1$), oral allergy syndrome ($n = 1$), asthma ($n = 2$), food allergy ($n = 2$), allergic rhinitis ($n = 6$), and eosinophilic esophagitis ($n = 9$). No attempt was made to distinguish salivary cytokine profiles between children with and without allergies in this study.

Participants took nothing by mouth for at least 4 hours. They rinsed their mouth with water, and after a 10-minute period, passive drool (PD) was collected followed by saliva collection via an oral swab (OS) to minimize the effect of saliva on the flow rate and concentration of cytokines in PD. The PD samples were obtained with the head tilted forward while drooling down a 5.5-cm polypropylene collection aid (Salimetrics Collection Aid; Salimetrics LLC, State College, Pennsylvania). Next, the participant placed an OS (Salimetrics Oral Swab; Salimetrics LLC) in their mouth and gently rolled it side to side for approximately 60 to 90 seconds to saturate the swab, which was then placed in a storage tube that allows saliva

to be centrifuged. All samples were collected between 7:30 AM and 12:30 PM. Two participants (9%) aged 11 and 14 years were unable to provide PD because they were not able to direct their drool into the collection device. All participants were able to use the OS.

After providing paired saliva samples, all children responded to a short questionnaire assessing their knowledge of salivary diagnostics, asking them to rate their experience in providing each saliva sample in terms of perceived ease of use, acceptability, and safety. Only 23% were aware of saliva testing in the surveillance of health or disease. A significantly higher proportion of children preferred the OS over PD sampling (82% vs 18%; $P < .05$). Ease (68%) and speed (14%) of providing samples were the most common reasons cited by those who preferred the OS.

Saliva samples were maintained at 4°C and transported to the laboratory within 2 hours of collection and were centrifuged at 1,650 g for 15 minutes. Supernatants were stored in aliquots and frozen at –80°C. On the day of analysis, samples were brought to room temperature, vortexed, and centrifuged for 15 minutes at 1,650 g. A magnetic, high-sensitivity, human multianalytes profiling, bead-based assay kit was used (EMD Millipore, Billerica, Massachusetts; lower limit of detection and coefficient of variation for IL-4, IL-5, and IL-13, 0.13 pg/mL and <10%, <6%, and <8%, respectively; lower limit of detection and coefficient of variation for Eo3 and TSLP, 3.2 pg/mL and <9% and <12%, respectively). All cytokines were measured on 96-well plates (50 μ L of saliva per well). The standards and controls were plated on every run using a saliva-based sample matrix. Plates were read on the Luminex 200 platform (Luminex, Austin, Texas) according to the manufacturer's recommendations. Intra-assay precision was performed using 10 samples in duplicates, and the coefficient of variation was calculated.

IL-4, IL-5, and IL-13 were detected in all PD and OS samples. Eo3 and TSLP were undetectable in 3 PD samples (17%) but were detectable in all OS samples. The Wilcoxon rank sum test revealed that the median concentrations were similar between PD and OS samples (Table 1); however, greater precision was noted in OS compared with PD concentrations. The Bland-Altman analysis⁵ revealed no systematic bias and insufficient agreement between PD and OS, suggesting that PD and OS may not be used interchangeably.

To our knowledge, this is the first study to describe the presence of cytokines related to allergic inflammation in saliva in a pediatric population. Our results suggest that OS provides more precise concentrations and is more acceptable than PD for detection and analysis of cytokines related to allergic inflammation. Furthermore, our data suggest that PD and OS may not be used interchangeably. Our data extend previous findings indicating that saliva collection method can affect the salivary concentrations of stress and nonallergic inflammatory markers.^{6–9}

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Table 1

Salivary concentrations of cytokines associated with allergic inflammation in PD and saliva collected in OS

Cytokine	Median (IQR), pg/mL		P value
	PD	OS	
IL-4	27 (21–45)	29 (22–38)	.37
IL-5	1.5 (0.8–3.6)	1.4 (0.9–2.0)	.36
IL-13	4.5 (2.5–10.1)	4.8 (3.1–5.8)	.39
Eo3	52.7 (44.1–60.3)	45.6 (41.5–55.2)	.15
TSLP	7.4 (7.0–7.8)	7.2 (6.9–7.4)	.15

Abbreviations: Eo3, eotaxin 3; IL, interleukin; IQR, interquartile range; OS, oral swab; PD, passive drool; TSLP, thymic stromal lymphopoietin.

Strengths to our study included the ability to minimize the effects of external factors, such as tooth brushing and recent meals by collecting saliva samples from children who took nothing by mouth for an acceptable period before providing saliva samples. We were successful in collecting saliva samples (both PD and OS) from children as young as 6 years.

Limitations of our study include our use of a small convenience sample. In addition, our focus was on cytokines relevant to allergic inflammation. It is possible that either PD or OS may be acceptable for other biomarkers. The presence of eosinophils, eosinophil degranulation products, and/or epithelial cells and their influence on the concentrations of specific cytokines in PD or OS remains unclear.

In summary, in the pediatric population, saliva collected by OS appears to offer methodologic advantages over PD in analyzing cytokines related to allergic inflammation. Adequately designed studies to evaluate the potential of salivary cytokines as noninvasive, point-of-care markers to diagnose and/or manage common allergic conditions that affect children are warranted.

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