Published in final edited form as:
Psychoneuroendocrinology. 2021 February ; 124: 105069. doi:10.1016/j.psyneuen.2020.105069.

Measuring salivary markers of inflammation in health research: A review of methodological considerations and best practices

Yvette Z. Szabo[a,b,1], **Danica C. Slavish**[c]

[a]Department of Veterans Affairs VISN 17 Center of Excellence for Research on Returning War Veterans, Waco, TX, USA

[b]Department of Health, Human Performance and Recreation, Baylor University, Waco, TX, USA

[c]Department of Psychology, University of North Texas, Denton, TX, USA

Abstract

There is an increasing interest in using saliva to measure inflammatory biomarkers. Compared to blood, saliva is non-invasive, requires a lower biosafety classification, and requires less specialized personnel to collect. As the assessment of inflammation in saliva becomes more popular in psychoneuroimmunology research, the development of gold-standard methodological practices is paramount. This paper reviews different considerations for designing studies to assess salivary measures of inflammation. We review saliva collection procedures, sample storage and processing considerations, assay techniques, flow rate, correspondence with blood-based markers, and potential demographic and health moderators of levels of salivary markers of inflammation. Together, this review highlights critical gaps for future research, including calls for standardization of study protocols, transparent reporting of results, assessing predictive validity of markers of salivary inflammation for disease, and the need for assessment of participants' oral and general health status. Although additional work is needed to elucidate gold standards for study design, measurement, and analysis, salivary markers of inflammation may be a useful tool for understanding oral and peripheral inflammation dynamics non-invasively.

Keywords

design; measurement; analysis; saliva; non-invasive; cytokine

Inflammation is a biological process that has broad implications for mental and physical health (Dowlati et al., 2010; Kendall-Tackett, 2010; Michopoulos et al., 2017; Miller & Blackwell, 2006). There is an increasing interest in using saliva to measure inflammatory biomarkers, as it is non-invasive, requires a lower biosafety classification compared to blood, and requires less specialized personnel to collect. This can be particularly helpful for collection of samples from vulnerable populations, such as children (Pappa et al., 2019), older adults, or individuals who otherwise might not donate blood samples due to needle phobia or anxiety. Further, the concentration of detectable levels of inflammatory markers in saliva is relatively high (Byrne et al., 2013; Shields et al., 2019; Szabo et al., 2016),

[1] Corresponding Authors: Yvette Z. Szabo, 4800 Memorial Drive (151C), Waco, Texas 76711 (254) 297-3179; yvette.szabo@va.gov.

sometimes higher than the concentration detected in blood (Byrne et al., 2013), highlighting the unique utility of saliva for assessing inflammatory markers.

There have been some reservations expressed regarding the use of saliva to measure inflammation due to potential confounds, such as oral health, and inconsistent associations with blood-based markers (Wilson et al., 2018). Recent reviews (Slavish, Graham-Engeland, Smyth, & Engeland, 2015; Slavish & Szabo, 2019; Szabo, Slavish, & Graham-Engeland, 2020) and opinion papers or chapters (Engeland, Bosch, & Rohleder, 2019; Riis, Byrne, Hernández, & Robles, 2020) point to a need to evaluate the validity of salivary markers of inflammation (i.e., both how they relate to systemic inflammation and health-relevant phenomena). Despite these possible limitations, salivary markers of inflammation have been shown to be associated with or have validity for mental health conditions (Newton et al., 2014; Quinn et al., 2020), physical health disorders (Chauhan et al., 2016; Ebersole et al., 2017; Gohel et al., 2018; Out et al., 2012; Silvestre-Rangil et al., 2017), and psychological phenomena like trauma exposure, perceived stress, and emotion (Buzgoova et al., 2019; Jabber, Zaidan, Gorial, & Al-Naaimi, 2015; Slavish et al., 2019; Szabo, Fernandez-Botran, & Newton, 2019; Tell, Mathews, Burr, & Janusek, 2018).

As the assessment of inflammation in saliva becomes more popular in psychoneuroimmunology research, the development of gold-standard methodological practices is paramount. Previous reviews and chapters have provided guidelines for collecting saliva to assess other biomarkers, including cortisol and alpha amylase, or salivary biomarkers more broadly (Granger et al., 2012; Padilla et al., 2020; Shirtcliff et al., 2001). Unique considerations for the collection and assessment of inflammatory markers warrant a separate review. The purpose of this paper is to provide readers with an easy to use guide for the collection and analysis of saliva for measurement of salivary markers of inflammation in research settings based on the current state of the field. For a broader guide on salivary biomarkers, we direct readers to a recent book that discusses salivary markers for use in interdisciplinary research (Granger & Taylor, 2020).

Overview of salivary markers of inflammation

Whole saliva is derived primarily from three major salivary glands: the parotid, submandibular and sublingual glands, as well as from minor salivary glands in the oral mucosa (Pedersen, Bardow, Jensen, & Nauntofte, 2002). Whole saliva also contains gingival crevicular fluid (GCF), microorganisms from dental plaque, and food debris (Subbarao et al., 2019). Some common markers of inflammation measured in saliva include cytokines (e.g., interleukin [IL]-1β or tumor necrosis factor [TNF]–α) or acute phase proteins (e.g., C reactive protein [CRP]).

Acute Phase Proteins

CRP is an acute phase protein secreted by the liver (Kindt et al., 2006; Marnell et al., 2005), and promotes the production of pro-inflammatory cytokines in response to infection (Du Clos, 2000). CRP activates the complement cascade, which helps mediate protection from bacteria (Marnell et al., 2005). Because CRP is produced only in the liver and enters

saliva through processes such as passive diffusion or filtration from other oral fluids (e.g., GCF), CRP levels in saliva are often lower than levels in blood (Kopanczyk et al., 2010). Because salivary CRP is not synthesized locally in the mouth, it may reflect more systemic levels of inflammation compared to other inflammatory biomarkers (e.g., cytokines). As shown in Table 1, there is variability in the magnitude of correlations of salivary and blood-based measures of CRP across studies with healthy and clinical samples (r_s = −.17 to .73). Further, there is some evidence that the correlation is strongest in individuals with high levels of CRP, but others have found strong associations when removing individuals with high CRP. Importantly, salivary CRP levels predict cardiovascular disease (Out et al., 2012) and myocardial infarction (Ebersole et al., 2017), and therefore may be a particularly health-relevant biomarker of inflammation.

Another such marker is the acute phase protein fibrinogen, which, like CRP, is synthesized in the liver (Tennent et al., 2007); thus, levels of fibrinogen in saliva reflect blood proteins. Fibrinogen levels may be low in saliva (De Oliveira et al., 2017), at least lower than levels in blood (Helmi et al., 2016; Hirtz et al., 2016). One study reported that levels of salivary and serum fibrinogen are uncorrelated in healthy controls (R^2 = .05) but significantly correlated in women with polycystic ovarian syndrome (R^2 = .50) (Helmi et al., 2016). Salivary fibrinogen has some diagnostic potential for tuberculosis (Jacobs et al., 2016) and levels are higher in individuals with polycystic ovarian syndrome compared to controls (Helmi et al., 2016).

Cytokines

Cytokines are small, cell signaling proteins that play role in inflammation and are secreted in response to infection or injury, with either primarily pro-inflammatory (e.g., TNF-α, IL-1β) or anti-inflammatory (e.g., IL-4, IL-10) functions. Some cytokines (e.g., IL-6, IL-1β, TNF-α) are produced locally in the mouth and also filter into whole saliva from a variety of sources, including the salivary glands, gingival fold, oral mucosa transudate, and mucus from the nasal cavity (Desai & Mathews, 2014). While some studies have reported levels of many cytokines are lower in saliva than blood (Nam et al., 2019; Riis et al., 2014), there is a growing literature suggesting some markers, such as levels of salivary IL-1β, may be higher than in blood (Nam et al., 2019; Riis et al., 2014; Szabo et al., 2016). Though not exhaustive of all cytokines measured in saliva, Table 1 reviews correlations between blood and saliva for some of the most commonly studied markers. As shown in Table 1, correlations between salivary and blood-based IL-6 range between r = .07 to .71. Smaller correlations have been reported for each IL-1β (r = .01-.11) and TNF-α (r = −.15-.32). For each IL-10 and IFN-γ, correlations with blood are lower in healthy adults (IL-10 =.21-.28, IFN-γ = .33-.34) compared to clinical samples (IL-10 = .59-.86; IFN-γ = .78). In terms of correlations after stress, changes in salivary IL-6, IL-1β, IL-18 pre to post exam stressor significantly correlate with changes in plasma: IL-1β = .53, IL-6 = .45; IL-18 = .59 (La Fratta et al., 2018), and peak levels of plasma IL-6, IL-10, IL-4, IL-8 correlate with peak levels saliva after an experimental pain paradigm: IL-6 = .61, IL-10 = .62, IL-4 = .51, IL-8 = .61 (Cruz-Almeida et al., 2017).

A recent review of salivary markers of inflammation in response to an acute stressor evaluated 17 biomarkers (CRP, granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-21, interferon (IFN)-α, IFN-γ, and TNF-α) (Szabo, Slavish, & Graham-Engeland, 2020), with the majority of these markers reported as detectable in saliva across multiple studies. Some of these markers have predictive validity for mental and physical health conditions. Levels of salivary IL-8 and TNF-α predict myocardial infarction (Saraev et al., 2019), and the combination of IL-1β and matrix metalloproteinase (MMP)-8 may serve as biomarkers of periodontal disease (Miller, King, Langub, Kryscio, & Thomas, 2006). Veterans with PTSD exhibit higher levels of salivary IL-2, IFN-γ, IL-6, IL-17, and lower IL-4 and IL-10, compared to controls without PTSD (Wang et al., 2016). Together, these studies suggest many markers of inflammation are measureable in saliva and appear to correlate with mental and physical health status.

Methodological Considerations

Time of Day

Timing of sample collection is important to consider, as many cytokines measured in blood have a circadian rhythm (Coogan & Wyse, 2008). For example, IL-6 tends to have two peaks around 5am and 7pm, with two nadirs around 8am and 9pm (Vgontzas et al., 2005). IL-1β also appears to exhibit two peaks around 3am and 5pm, with a nadir around 10am (Cuesta et al., 2016). Blood levels of IFN-γ, TNF-α, IL-2, and IL-12 also appear to show similar patterns, with a peak generally observed at night or in the early morning (Cermakian et al., 2013). In contrast, blood-based CRP does not appear to have a strong diurnal rhythm in healthy adults (Meier-Ewert et al., 2001). It is unclear if these studies in blood map onto the circadian rhythm of salivary markers. One study showed that like blood, salivary IL-6 levels peaked at awakening, gradually declining from morning to noon, and peaking again at midnight (Izawa, Miki, Liu, & Ogawa, 2013). However, unlike blood-based CRP, salivary CRP may have a circadian rhythm, with levels peaking at awakening, and declining throughout the daytime (Izawa, Miki, Liu, & Ogawa, 2013), although other research has suggested the circadian rhythm may depend on data cleaning decisions (e.g., Winsorizing out of range values) (Landau et al., 2019). We recommend that samples are collected at the same time for all participants whenever possible or that authors statistically control for sample collection time or time since waking in analyses.

Values at rest and in response to stimuli.

Another study design consideration would be to determine whether salivary markers of inflammation are measured at rest or in response to stimuli (e.g., a stressor, exercise, or cognitive task). Several salivary inflammatory markers increase in response to acute stress. For example, a recent meta-analysis showed that salivary cytokines IL-6, IL-10, TNF-α, and IFN-γ significantly increased in response to acute social and exercise stressors, with small to moderate effect sizes. After removing outliers, IL-1β and IL-8 also increased in response to acute stressors, but IFN-γ did not (Szabo, Slavish, & Graham-Engeland, 2020). When examining salivary markers in response to acute stressors, length of exposure to stimuli and timing of sample assessments pre- and post-stress are also important considerations. For

example, in the meta-analysis described above, for IL-6, the largest effect size was found an average of 47.86 minutes after the start of the stressor; for IL-10, 98.68 minutes after the start of the stressor; for TNF-α, 66.52 minutes after the start of the stressor; and for IFN-γ, 65.00 minutes after the start of the stressor, with large variability from study to study. Together, these findings suggest it may take between approximately 45 minutes to 100 minutes after the initiation of the stressor to observe peak salivary cytokine responses, depending on the biomarkers of interest. Few studies have measured saliva at multiple times during an acute stressor paradigm (e.g., Goetz & Lucas, 2020; Izawa et al., 2013b; Kimura et al., 2013; Tell et al., 2018), suggesting the need for more careful examination of the time course of salivary inflammatory biomarker release following acute stress exposure.

When measuring salivary markers of inflammation in response to some sort of task or stimuli, we also encourage the use of resting baseline samples and validated experimental paradigms whenever possible. A resting baseline sample (i.e., having the participant rest quietly prior to taking the first sample) would help reduce the potential confounding role of anticipatory stress due to the research protocol or variability in baseline mental state prior to the research session. Theoretically, a resting sample allows researchers to more accurately capture the response to the stimulus by reducing heterogeneity in baseline responses. Highlighting the importance of anticipation, one study showed levels of salivary IL-6 were highest among individuals with PTSD prior to a research visit that included a trauma interview (Newton et al., 2014). La Fratta and colleagues (2018) measured IL-1β three times: 1) at rest during a non-exam period of the semester, 2) before an exam, and 3) immediately after an exam. They showed that levels of IL-1β and IL-18 were significantly higher before the exam compared to the rest day and decreased during the course of the examination. However, the same pattern was not found for IL-6 or CRP (La Fratta et al., 2018). Other research has suggested the role of anticipation may be most important for events that are personal, such an exam or a trauma interview, compared to a laboratory stressor (Riis, Ahmadi et al., 2020). Due to concerns about stability of salivary markers of inflammation (see below), to enhance reliability, an alternative might be to take multiple baseline samples (e.g., one sample two hours before a stressor and another sample immediately before a stressor), which is an approach that has been used in some research (e.g., Minetto et al., 2005).

In terms of validated experimental paradigms, most research uses acute stress paradigms (e.g., the Trier Social Stress Test) (Kirschbaum et al., 1993) that were validated to induce stress and other physiological or emotional responses for different biomarkers (e.g., cortisol). Additional validation is needed to see if these paradigms also induce changes in salivary markers of inflammation.. Further, using control groups and cross-over designs (where the same individuals are exposed to both stressor and non-stressor conditions) would allow for inferences about whether observed effects were truly the result of stress exposure, opposed to individual differences.

Potential Use of Saliva in Naturalistic Settings

Given the relatively non-invasive nature of saliva, it is ideal for collection in naturalistic settings to inform how salivary markers of inflammation relate to changes in daily processes.

For example, one study assessed associations between daily work and sleep schedules with changes in salivary IL-1β, TNF-α, and IL-6 over multiple time points across a day in a naturalistic setting (Reinhardt et al., 2019). This study showed that both day and night workers exhibited similar daily variation in salivary IL-1β and TNF-α, but differences in patterns of daily IL-6, particularly upon awakening. A similar study showed that short sleep duration is associated with elevated salivary IL-6 among shift workers (Reinhardt et al., 2016). Higher levels of daily positive affect across 14 days has also been linked to lower levels of salivary CRP (Slavish et al., 2019). These initial results suggest salivary biomarkers may be responsive to daily fluctuations in sleep and mood, and highlight feasibility of their use in more naturalistic settings. Using salivary assessments of inflammatory markers instead of blood allows for a greater number of samples from the same individuals, enhancing potential understanding within-person fluctuations across time.

There are some unique considerations for saliva collection in naturalistic settings, such as short-term storage and handling of samples, and how to monitor participant compliance to saliva collection protocols. Participants should be given clear instructions about how to collect samples properly, including behaviors to avoid before sample collection, how to record time of sample collection (e.g., electronic diary, handwritten log, scanning a QR code or taking a time-stamped photo), and how to store samples temporarily. For ease of collection, it may be beneficial for researchers to create individually labeled daily packages of saliva collection materials (e.g., saliva collection aids, instructions, log to record sample timing) for each day of expected saliva collection. When participant transport of saliva samples back to the lab or retrieval by researchers is not feasible, procedures and equipment for mailing saliva samples to the appropriate location should be carefully discussed with participants (e.g., mailing samples on dry ice in a travel cooler).

Participant Characteristics

Oral Health Status

Individual differences in participant characteristics may contribute to variability in levels of salivary markers of inflammation. As outlined by Engeland and colleagues (2019), individuals with good oral health should theoretically have levels closer to systematic inflammation (e.g., blood- based markers), as poorer oral health would be indicative of sources of local inflammation production. However, oral health is often correlated with mental and physical health (Janket et al., 2003; Matevosyan, 2010). Therefore, statistically controlling for oral health may be “overly corrective” when examining psychophysiological correlates of inflammation.

Another consideration is that cytokines and other inflammatory markers can filter into the saliva from peripheral systems through leaky patches in the oral mucosa (Bosch, 2014). Individuals with leaky patches may therefore have stronger correlations between salivary and blood based markers. Given preliminary evidence that levels of salivary acute phase proteins CRP and fibrinogen are more strongly correlated in individuals with higher levels of inflammation or those with inflammatory-related diseases (Dillon et al., 2010; Helmi et al., 2016; Out et al., 2012), saliva may be a more justified method of measurement in these

populations. Further research is needed, but in non-clinical samples, oral health may be a more relevant confound, due to less shared variance between levels in blood and saliva.

Underscoring the importance of the oral cavity, in one study, salivary cytokine levels in adolescent females correlated strongly with salivary adiponectin (*rs* = .46-.77), a hormone that regulates glucose levels, whereas serum cytokines did not (*rs* = −.08–00) (Riis et al., 2014). Individuals with an acute myocardial infarction had both higher salivary CRP and fewer teeth and poorer oral health compared to controls, suggesting a potential correlation between the oral environment and other peripheral systems that may impact salivary CRP (Ebersole et al., 2017). A small but significant correlation between toothache/dental pain and salivary CRP was observed among women with intimate partner abuse histories (*r* = .21) (Out et al., 2012). In one recent study of healthy young adults, higher levels of salivary IL-6, IL-8, IL-1β, and TNF-α were strongly associated (*rs* = .44 to .75) with poorer oral health (i.e., greater blood leakage, as measured by transferrin levels, and greater potential tissue degradation, as measured by levels of MMP-8) (Riis, Byrne, et al., 2020). Together, this evidence suggests higher levels of salivary markers of inflammation are associated with poorer oral health.

The determination of oral health status of participants will be important for elucidating potential confounds. In addition to increases in inflammation due to poor health, blood leakage into saliva due to gingivitis could result in increased concentrations of salivary measures that are higher in blood compared to saliva (Kamodyová et al., 2015). However, this is not as clear for cytokines, some of which are often higher in saliva than in blood. Oral examination by a trained clinical is likely costly and impractical for most research settings. Thus, the inclusion of questions regarding oral health is recommended whenever assessing salivary markers of inflammation. Brief validated screening questions that inquire about dental cavities (e.g., caries), deep pockets or receding gums, and periodontal or gum disease have high sensitivity and specificity when compared to a clinical exam (LaMonte et al., 2014; Buhlin et al., 2002; see Table 2). In addition to questions like these, researchers may also benefit from inquiring about participants' recent dental procedures; sensitive, painful, or bleeding gums; and tooth pain. Given the high prevalence of oral health problems in U.S. adolescents and adults (42–57% for periodontitis or dental caries) (Centers for Disease Control and Prevention, 2019; Eke et al., 2018), it may be worth statistically examining the impact of poor oral health status, rather than automatically excluding individuals on this basis (if researchers are seeking to generalize to the population level). Although analytic decisions depend on the research question of interest, it may be worthwhile for researchers to report correlations between oral health and levels of salivary markers, run sensitivity analyses excluding those with poor oral health, and/or create a latent or composite variable of oral health measures. As the literature examining these factors expands, more definitive best practices can be established.

Other Health Conditions

A previous review on the measurement of inflammatory markers in blood outlined several health-related factors to assess (e.g., diet, smoking, medication use), control for (e.g., age, sex, body mass index), or exclude participants based on (e.g., acute exercise,

sleep deprivation, chronic smoking or alcohol dependence (O'Connor et al., 2009)). Although it is likely some of these same recommendations apply to salivary markers of inflammation, further work in this area is needed. As a preliminary step, in alignment with recommendations from O'Connor et al. (2009) and existing research, we outline some similar considerations for researchers assessing salivary markers of inflammation in Table 2. It may be worthwhile to evaluate or potentially exclude individuals with autoimmune diseases or diseases that could impact salivary production (e.g., Sjögren's syndrome, Crohn's disease, systemic lupus erythematosus, anorexia nervosa or bulimia, burning mouth syndrome) (Pedersen et al., 2002). These individuals may have altered or atypical immune responses and/or decreased salivary flow rate, each of which may impact concentrations of circulating salivary inflammatory markers.

In terms of demographic influences on salivary markers of inflammation, a recent meta-analysis found that studies with a greater proportion of racial or ethnic minority participants had greater post-stress increases in salivary IL-1β, but not other biomarkers (e.g., IL-6, CRP, TNF-α) (Szabo, Slavish, & Graham-Engeland, 2020). Though limited by sample size and study homogeneity, the same meta-analysis did not find significant moderation of stress-related responses in salivary biomarkers by other demographic factors, such as age, or gender, or health status (clinical vs. healthy vs. mixed sample). However, one small study (n =15)found older adults have greater IL-6, IL-10, IL-8 and IL-4 responses to experimental pain paradigms than younger adults at 45–60 minutes post manipulation (Cruz-Almeida et al., 2017), suggesting these demographic characteristics may still be important to consider.

With regard to health behaviors and characteristics, a recent review suggested smoking impacts levels of salivary IL-8, but not IL-1β or IL-6 (Jaedicke et al., 2016). Nicotine triggers neutrophil activation, which can lead to inflammatory cytokine release and oxidative stress (Hosseinzadeh et al., 2016). Chronic inhalation of cigarette smoke also causes tissue damage (Van Der Vaart et al., 2004) and can increase risk for poor oral health and chronic diseases associated with systemic inflammation (e.g., cardiovascular disease) (Calsina et al., 2002; McEvoy et al., 2015). In a longitudinal observational study of adolescent girls, cytokine levels did not vary by smoking status at baseline, but IL-1β was higher in smokers in year 2, and IL-1β, IL-2 and IL-6 levels were higher in smokers in year 3 compared to non-smokers (Riis et al., 2014). Findings with CRP vary; a longitudinal study of women with histories of intimate partner abuse reported no difference in salivary CRP between individuals who did or did not smoke (Out et al., 2012), but another study of healthy young adults found a linear, dose response association between salivary CRP and smoking, such that as smoking levels increased, so did CRP levels (Azar & Richard, 2011).

In sum, there are several participant characteristics that may impact levels of salivary markers of inflammation. Despite inconsistent associations, we still encourage assessment of these characteristics and behaviors for use as potential covariates (see Table 2). Many of these previous studies did not specifically aim to evaluate these demographic variables, and they are preliminarily associated with some salivary markers and of theoretical importance. Furthermore, adjustment for covariates may affect correlations between salivary and serum markers of inflammation. For example, after adjusting for age, smoking status and an oral health proxy, levels of serum IL-1β were significantly positively correlated with salivary

IL-1β (Riis et al., 2014). This finding points to the need for careful consideration of potential confounds to improve comparison between salivary and blood markers.

Restrictions on behaviors prior to saliva collection

Saliva collection procedures should also be carefully considered by researchers. For example, to remove food related debris, some research recommends participants rinse their mouth with water prior to saliva collection, and then wait 10 minutes before collecting saliva to reduce the potential for dilution (Whembolua et al., 2006). This procedure has been used in several salivary cytokine studies (e.g., Newton et al., 2017; Slavish et al., 2019). Other studies have provided water to drink upon arrival to the laboratory and after each saliva sample (Laurent et al., 2016), which may help stimulate saliva production and remove debris. We also urge researchers to have participants restrict food intake and teeth brushing at least one hour prior to sample collection to eliminate the potential for debris or blood in the sample. Caffeine and alcohol should also be avoided prior to collection. The half-life of caffeine is approximately 5–6 hours, and the ethanol elimination rate is between 15–18 mg/dL per hour. Therefore, we recommend researchers have participants restrict their caffeine and alcohol use for at least 3 hours before sample collection. Since exercise has been shown to increase levels of salivary markers of inflammation (Minetto et al., 2007; Minetto et al., 2005), we also recommend having participants avoid exercising for at least one hour prior the study procedure. Several previous studies have provided these or similar guidelines to their participants (e.g., Auer et al., 2018; Szabo et al., 2019).

Saliva Collection Procedure

To collect salivary markers of inflammation, investigators must invest in materials, including saliva collection devices, gloves, a cooler or freezer for sample storage, and institutionally recommended biohazard containers, particularly in case of any noticeable blood contamination in saliva samples. For those in a laboratory setting, local institutions may require biosafety classification or other specialized trainings to collect biological samples, as well as a dedicated space conducive to collecting saliva samples (e.g., space with a tile floor and sink). For processing, researchers may need a centrifuge, pipettes, and freezer safe tubes to store aliquots.

Saliva collection procedures can impact the ability to obtain reliable and valid estimates of inflammation. Some salient factors for researchers to consider include participant behaviors to restrict or measure prior to saliva collection and the specific saliva collection methods used; each factor is discussed below and displayed in Table 2.

Saliva collection methods

There are multiple methods that can be used to collect saliva; the below sections synthesize recent research on their use in research on salivary markers of inflammation (for a review on saliva collection methods more generally, please see Bellagambi et al., 2020).

Passive drool.

Passive drool is considered the "gold standard" of saliva collection and involves passively allowing whole (sometimes called "mixed") saliva to pool in the mouth and then flow through a plastic straw into cryovials for a specific amount of time (e.g., 1 or 5 minutes). Compared to spitting, passive drool avoids more localized secretion from specific salivary glands and provides a more consistent sample. To encourage saliva flow, many researchers use prompts or scripts. For example, researchers may encourage participants to imagine lemon, state the word "lemon" (Padilla et al., 2020), ask participants to move their jaw like they are chewing (Granger et al., 2012), or think of a favorite food to allow saliva to pool at the bottom of the mouth (Auer et al., 2018; Granger et al., 2012). Having the participant close their eyes or tip their head forward can facilitate saliva collection and reduce the likelihood of swallowing saliva. Sufficient saliva volume collection can be particularly difficulty in early childhood or geriatric populations; allowing for additional time or planning for smaller volumes can be helpful to mitigate missingness in these populations (Granger et al., 2007).

When using passive drool methods, researchers should consider the volume needed for analyses. Most assay kits need 10 to 25 μL per well and analyze samples in duplicate or triplicate. Therefore, it is recommended that researchers collect at least 200 μL for each assay they expect to run. A recent chapter focused on salivary biomarkers for interdisciplinary research suggests adding the amount anticipated for markers of interest plus 300 mL as a buffer when examining multiple analytes (Padilla et al., 2020).

We also recommend researchers calculate salivary flow rate (discussed further in the section "Flow rate and other adjustments to inflammation concentration in saliva"). Salivary flow rate can be calculated by subtracting the pre-weight measure of the collection vial (e.g., in grams) from the post-weight measure of the collection vial (e.g., in grams), divided by the length of collection time (e.g., 5 minutes), which results in a rate of how much saliva was collected across the collection period (e.g., grams per minute). Salivary flow rate may depend on whether stimulated or unstimulated samples are used. For example, one study showed having participants chew on a piece of silicone tubing for one minute produced significantly higher salivary flow rates when compared with unstimulated resting drool (Mohamed et al., 2012).

Spitting.

Other methods to collect saliva include spitting in a collection tube to a certain volume. This can be differentiated from passive drool methods, as spitting involves actively expelling saliva from the mouth into a tube (opposed to letting it passively flow into a tube). Spitting allows for easier collection of saliva but can be messy and discourage participant donation (Salimetrics, 2020b). Spitting also encourages saliva production from specific salivary glands, which may provide a less comprehensive assessment of salivary levels of inflammation than passive drool methods. As such, this is not a recommended method.

Swab- or sponge-based methods.

Another common method to collect saliva includes the use of a cotton (e.g., a Salivette) or synthetic swab or sponge (e.g., SalivaBio Oral Swab). Because these methods involve chewing on cotton or resting a swab against the gums, these methods can be particularly helpful with children to facilitate ease of collection (Pappa et al., 2019). Some of these devices are also available with citric acid coating to facilitate saliva production. Some research has suggested that some, but not all, immunoassays are impacted by cotton based collection methods (Shirtcliff et al., 2001). We discourage the use of traditional Salivettes or other cotton swabs due to research suggesting that that the use of a cotton swab impacts inflammatory biomarker level. For example, Minetto and colleagues compared saliva collected with Salivette versus spitting and reported a mean difference between the two measurements of nearly 4 pg/mL in resting IL-6 (Minetto et al., 2007), concluding that there is a cotton interference effect for salivary IL-6. Another study showed that compared to passive drool collection, median concentrations of salivary IL-6, IL-8, IL-1β, and TNF-α were lower when using either an ultra-light (0.05 g/cc) or high-density swab (0.077 g/cc), although this difference was not statistically significant for IL-1β (Riis, Ahmadi et al., 2020). More recently, a synthetic, non-cotton Salivette has been developed for use with cortisol, and some research has started to use a non-cotton sponge for collection of saliva for measuring inflammation (e.g., Reed et al., 2017). These approaches may offer some additional benefit, by reducing the issue of cotton interference.

Stimulated saliva.

Stimulated saliva, or saliva collected from mechanical production is another approach for collecting saliva. This can be done by chewing on paraffin wax (Bellagambi et al., 2020) or silicone tubing (Mohamed et al., 2012), then expectorating saliva until the desired volume is collected. Stimulated saliva has been used to measure CRP, and some research recommends this collection approach (in combination with not centrifuging samples prior to long-term storage) in order to maintain biomarker levels (Mohamed et al., 2012). However, it is unclear if these same recommendations extend to other inflammatory markers. Given the possibility for debris contamination, and evidence from studies in other biomarkers(e.g., Riis, Ahmadi et al., 2020), our current recommendation is to use unstimulated passive drool sample and centrifuge samples prior to long term storage to remove potential debris.

Filter paper.

Filter paper is another saliva collection option, and involves putting a piece of filter paper in the sublingual pocket of the individual’s mouth until the paper becomes saturated (Williamson et al., 2012). Similar to flow rate, the extent to which the paper is saturated can indicate volume of fluid secreted to produce the measured concentration of inflammatory marker. Although this may facilitate ease of collection and allow for sampling of specific minor glands (Bellagambi et al., 2020), this approach is not widely used, and future research is needed to test whether this approach impacts levels of inflammatory markers. One study used filter paper and passive drool to collect saliva and assayed a panel of 27 cytokines. No significant correlations were observed for the 27 cytokines measured in saliva collected with

filter paper compared to plasma, and only levels of 16 out of 27 cytokines measured in saliva using the two methods showed significant correlations (Williamson et al., 2012).

Sample Handling and Processing

Short-term storage.

In some studies, researchers may be unable to immediately process saliva samples for long-term storage. In this case, it is critical to take steps to preserve sample integrity. Immediately after saliva sample collection, we urge researchers to refrigerate samples at 4°C, store on regular ice or dry ice, or store in a cooler with an ice pack until long-term freezing. This helps avoid sample degradation and bacterial growth (Whembolua et al., 2006). For example, one study showed storage of salivary IL-6, IL-8, IL-1β, or TNF-α at room temperature for one day results in significant reductions in median concentrations, with additional reductions for each additional day samples are stored at room temperature (Riis, Ahmadi et al., 2020). Researchers should avoid keeping samples at 4°C for longer than 2 hours (Salimetrics, 2020b). According to one study, storage at 4°C slows degradation, such that levels of salivary IL-8 remained relatively stable after 4 days at this temperature (Gröschl et al., 2008).

Centrifuging.

Samples should be centrifuged to remove insoluble material (e.g., mucous, food debris) and the remaining liquid (i.e., supernatants) collected by aspiration. Ideally, this would be done before storage, and again after thawing. Many studies report centrifuging samples for approximately 15 minutes at 1500 to 3000 revolutions per minute (rpm) (Izawa et al., 2013b; Minetto et al., 2007; Reed et al., 2017; Slavish et al., 2019). Salimetrics recommends centrifuging for 15 minutes at 1500 rpm (Salimetrics, 2020a). Regardless of what approach researchers take, we urge transparent reporting of processing methods (e.g., amount of time centrifuged and at what speed) to guide future research. We also encourage researchers to report centrifuge speed in relative centrifugal force (RCF, or G-force), instead of rpm. Unlike rpm, G-force is standardized across different centrifuges and can be easily calculated from rpm and centrifuge rotor radius using the following formula: = 1.12 x Radius of rotor (in mm) x $(rpm/1000)^2$.

Freeze/thaw cycles.

We also urge researchers to minimize freeze/thaw cycles by centrifuging and aliquoting samples into smaller vials upon collection, and then placing in a freezer for long term storage before assaying. In blood, IL-6 and IL-10 are stable throughout multiple freeze/thaw cycles, whereas markers IL-4, IL-13, IL-15, IL-17, TNF-α, IFN-γ and CXCL8 levels either rise (IL-4 and TNF-α) or drop (IL-13, IL-15, IL-17, IFN-γ and CXCL8) after one or more freeze/thaw cycles (de Jager et al., 2009). One recent study in saliva showed that compared to zero freeze/thaw cycles, four freeze/thaw cycles resulted in significantly lower median levels of salivary IL-6 and IL-8, but not IL-1β or TNF-α. Exposure to only two freeze/thaw cycles did not lead to significant reductions in IL-6, IL-8, IL-1β, or TNF-α (Riis, Ahmadi et al., 2020). Therefore, we recommend researchers avoid more than two freeze/thaw cycles when assessing inflammatory markers.

Long-term sample storage.

Long-term sample storage can impact levels of detectable salivary cytokines. Studies have stored samples long term at a variety of freezer temperatures, most commonly at −80°C, or at −20°C (Auer et al., 2018; Cruz-Almeida et al., 2017; La Fratta et al., 2018). In serum, biomarkers IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, TNF-α, and IFN-γ are stable up to 2 years of storage at −80°C, but after 4 years, several cytokines (IL-1α, IL-1β, IL-6, IL-10, IL-15) degrade 50–75% or more from baseline values (de Jager et al., 2009). Saliva samples can be stored at −80°C for several years; however, validation studies are needed, as the exact time has not yet been determined and may vary by analyte (Salimetrics, 2020a). One recent study showed storing salivary IL-1β, IL-6, IL-8, and TNF-α at either −20°C or 4°C for three months resulted in significant median decreases in levels of all biomarkers compared to storage at −80°C (Riis, Ahmadi et al., 2020).

Saliva samples collected via cotton or synthetic sponges can be frozen in the swab for up to 6 months with no decline in levels (Salimetrics, 2020b). However, if it is known ahead of time that samples may need to be stored for longer than 6 months, it is recommended that researchers express the saliva out of the swab, either by centrifugation or squeezing through a syringe immediately after collection, and storing the expressed saliva in cryovials, ideally at −80°C (Salimetrics, 2020b). Assaying several kits in large batches (e.g., batch assaying) — instead of assaying all samples at the end of the study — may be optimal for data quality when conducting studies across a long period of time.

Sample storage considerations for naturalistic settings.

When samples are collected in naturalistic settings, researchers should implement consistent procedures for sample storage. One study showed concentrations of biomarkers IL-1α, IL-1 RI, IL-1 RII, IL-1ra, IL-6, IL-18, IL-33, TNF-α, and IFN-γ are stable in serum at 4°C (i.e., the temperature of most household refrigerators) for up to 30 days (Vincent et al., 2019), with authors recommending of 3 days of storage for unseparated serum at 4°C for optimal concentration stability. Another study showed median concentrations of salivary IL-6, IL-8, IL-1β, or TNF-α degrade after just one day at room temperature (~20°C), and after 3 months at 4°C or −20°C (compared to storage at −80°C) (Riis, Ahmadi et al., 2020). However, it is unknown if salivary markers of inflammation are stable at 4°C or −20°C *up through* 3 months, as these authors only assessed levels at baseline and 3 months later. If researchers have participants return samples soon after collection (i.e., in person or without dry ice), storing in the refrigerator immediately should be advised to reduce freeze/thaw cycles. However, if there is an extended period of storage or shipment on dry ice, we recommend researchers instruct participants to immediately freeze samples in home freezers (which are typically −20°C).

Assay Considerations

Assay type.

A range of assay methods can be used for assessing salivary markers of inflammation. Researchers could send their samples to a company for commercial testing or can choose to conduct assays in-house. Researches will need to consider the benefits of each type of

test for the markers they wish to examine, and whether kits or assay procedures have been optimized specifically for saliva (vs. using a kit that was originally designed for blood-based markers). Each of these tests used to determine the level of the analyte being measured is a different types of immunoassays, which compare samples to a standard curve derived from known concentrations. The process of determining the concentrations of markers of inflammation within the sample can vary by technology. As an example, enzyme linked immunosorbent assays (ELISAs) are a plate-based assay technique that detect and quantify proteins in a fluid sample using antibodies that bind to a target antigen (Gan & Patel, 2013). The antibodies are linked to an enzyme, which produces a detectable color change if antibody-antigen binding occurs. This color change is then used to quantify the sample concentration of the target protein. Many commercial kits are available to measure cytokine concentrations by ELISA; most of these are considered single plex (i.e., each kit tests for one biomarker). One study validated the measurement of salivary IL-6 using ELISA (Minetto et al., 2005), and another validated the measurement of salivary CRP with an ELISA (Ouellet-Morin et al., 2011). Kits developed specifically for saliva are commercially available (e.g., Salimetrics).

There are several multiplex technologies available that build off ELISA principles to simultaneously test the concentrations of multiple analytes. Thus, the benefits of this technology are strengthened by high throughput and efficiency in testing multiple analytes. These tests include microbead arrays, which use internally color coded beads that bind with the analytes of interest, and non-bead based technologies that use electrochemiluminescence or other reactions for multiple specific capture antibodies (Leng et al., 2008).

One criticism of multiplex technologies is potentially reduced sensitivity. One study found correlations between serum-based markers IL-6, TNF-a, IL-17a, IL-2 varied by technology used (Yeung et al., 2016). Another study demonstrated high correlations ($r = 0.81$ to 0.94) between traditional single plex ELISAs and multiplex technologies (Luminex MAP®) for blood-based biomarkers IL-1β, IL-4, IL-5, IL-6, IL-10, IFN γ, and TNF α, but lower correlations for markers IL-12p70 ($r = 0.002$) and IL-13 ($r = 0.62$) (DuPont et al., 2005). At the time of this writing, there were not multiplex kits developed specifically for saliva, though many companies offer adaptations for saliva, which include diluting samples. However, one study validated the measurement of IL-1β and IL-8 in saliva using bead-based microarray technologies (Arellano-Garcia et al., 2008).

Validation of kits for use with saliva is an important direction for future research. The reagents and other kit materials are optimized for blood, which has a different composition and higher protein content than saliva. It is possible that these differences may impact the validity of the measurements. Further, given saliva can contain mucous and other supernatants, it is possible it could clog instruments designed for blood (described in Byrne et al., 2013). Finally, for cytokines that have lower concentrations in saliva than blood, it is possible that they may go undetected in saliva if a lower standard is not included.

Kit sensitivity.

Another consideration is sensitivity of the kit, or the lowest concentration of the marker the kit can detect. Given many inflammatory markers are observed at low levels in healthy

adults, this is a common issue for measures of inflammation in saliva, and inflammation research more broadly. High sensitivity kits have been developed, which have lower minimum detectable concentrations. These kits may be helpful, though they are not available for all salivary markers of inflammation. Alternatively, we recommend consulting with the companies about approaches to make kits more sensitive (i.e., diluting the standards). Some research suggests diluting saliva samples improves detectability of inflammatory markers, with 1:2 and 1:4 being the most commonly utilized dilutions for cytokines (Minetto et al., 2005; Reed et al., 2017), and some studies diluting up to 1:10 for CRP (Out et al., 2012). Other research has opted to conduct additional centrifuging to remove debris in saliva that may clog the assay (Byrne et al., 2013), which may be helpful for cytokines that have low concentrations. We recommend contacting companies manufacturing the kit for any standard operating procedures for adapting their kit to saliva or diluting samples, and using kits designed for saliva whenever possible.

Blood contamination assessment.

One additional concern when measuring levels of cytokines in saliva is samples may include traces of blood. Researchers may choose to ask additional questions indicating risk of blood in saliva, or run additional assays to assess possible blood contamination levels. The Blood Contamination in Saliva Scale is a single item, five-point scale that assesses blood contamination based on discoloration, and has high interrater reliability (Kivlighan et al., 2004). Two commonly assessed blood component markers are transferrin and hemoglobin. Assays specifically designed to detect blood contamination using transferrin or hemoglobin may be utilized. For example, saliva that contains 1–10% of hemoglobin demonstrates altered levels of salivary oxidative stress markers (Kamodyová et al., 2015). If blood is detected in saliva using these or other methods, we suggest researchers consider excluding these samples from their analyses. This approach has been used in some research (La Fratta et al., 2018), where individuals with salivary levels of transferrin ≥5 mg/L were excluded from analyses. Further research in relation to salivary markers of inflammation is needed. One study demonstrated small to large correlations with both transferrin and hemoglobin and levels of salivary CRP, IL-1β, IL-6 and TNF-α ($rs = -.16$ to .46) (Nam et al., 2019).

Intra-assay and inter-assay variation.

Most studies assay samples in duplicate, although triplicates may be an ideal benchmark. The greater the number of replicates, the more precise the measure, but this can become expensive for larger studies. We encourage researchers to always report inter- and intra-assay coefficients of variation (CV), to characterize the reliability of assay techniques.

In summary, there are many choices for researchers to make when selecting kits to measure cytokines, and these technologies are developing rapidly. Many researchers opt to ship samples for analysis at a laboratory or commercial testing facility. For those who choose to assay in house, we recommend consulting with a trusted collaborator or representative to help make these decisions, considering cost, lab equipment available, and research question (e.g., exploratory versus confirmatory, measure of interest), but above all, prioritizing the integrity of the test.

Flow rate and other adjustments to inflammation concentration in saliva

As the field continues to expand, research testing whether salivary flow rate impacts levels of salivary inflammation is an important area of inquiry. Under basal conditions, the rate of saliva production is approximately 0.5 mL per minute, with many studies reporting a range from .40 to .53 (Filaire et al., 2011; Nam, Kim, Chang, & Kho, 2019; Szabo et al., 2019, Szabo et al., 2016) and some research reporting higher rates (.89 to .90; Caris, da Silva, dos Santos, Tufik, & dos Santos, 2017). Preliminary evidence suggests flow rate is not impacted by time of day (Filaire et al., 2011). Some research suggests flow rate increases with acute psychosocial stress (Szabo et al., 2019), but decreases in response to exercise (Caris et al., 2017; Usui et al., 2011).

In terms of specific inflammatory markers, some cytokines appear influenced by flow rate, whereas others do not. For example, some research showed IL-1β and CRP levels were significantly and moderately correlated with flow rate (rs = −.30 to −.34), but IL-10, CRP and IL-6 were not (rs = −.12 to −.07) (Izawa et al., 2013b; Ouellet-Morin et al., 2011; Szabo et al., 2019). Demographic factors that influence flow rate are not well understood. Age may be one important consideration, as age increases the risk for chronic health conditions which may affect saliva production. One additional consideration may be medication use, as a common side effect of several medications (e.g., antihypertensives, antidepressants, analgesics, tranquilizers, diuretics, and antihistamines) is dry mouth (Guggenheimer & Moore, 2003; Närhi et al., 1999; Ship et al., 2002). Rinsing the mouth with water 10 minutes before collecting saliva may help facilitate saliva flow in individuals taking these medications.

Another consideration requiring future research is the level of total protein in saliva (e.g., the total amount of all proteins present in a saliva sample, including inflammatory proteins). Saliva is a watery fluid, which is derived from multiple sources, each of which may have different inflammatory or total protein concentrations. The level of total protein in blood has been reported to be 100-fold higher compared to saliva (Nam et al., 2019). The concentration of inflammatory markers per concentration of total protein (i.e., protein proportion of inflammatory markers) are higher in blood for IL-1β, IL-6 and TNF-α, but higher in saliva for CRP (Nam et al., 2019), suggesting protein level may influence concentrations. For IL-6, protein proportion levels in saliva correlated with levels in blood (r = .42; Nam et al., 2019) and a second study found that salivary CRP was more strongly associated with protein concentration (r = .62) compared to volume (r = .52)(Iyengar et al., 2014), though protein adjusted salivary CRP correlated slightly less strongly with serum than levels unadjusted for protein. One study reported levels of salivary IL-1β, IL-6 and IL-8 that were adjusted for protein level (Dogra et al., 2019). However, future research is needed to understand whether this improves predictive validity.

To calculate flow rate-adjusted salivary cytokine levels, researchers would multiply sample concentrations (pg/mL) by the corresponding salivary flow rates (mL/min), and results would be expressed in pg/min. Future research is urgently needed to determine whether levels of protein in saliva or flow rate reliably influence concentrations of inflammatory markers in saliva. Furthermore, should flow rate be determined to impact concentrations,

then potential confounds that may also affect flow rate, such as recent exercise, stress, medication use, and morbidity, could impact interpretation of inflammation levels in saliva. In addition, future research is needed to clarify whether levels unadjusted or adjusted for flow rate have stronger predictive validity for disease outcomes.

Reporting of Results

To facilitate replication of research and to inform future studies, we urge researchers to make their data openly available whenever possible, or to at least provide descriptive statistics (e.g., mean and standard deviation) of sample levels (and by time point). Studies should also be transparent about missing data and salivary inflammatory levels below detectable limits — a common issue when assessing salivary biomarkers. Often, these data are not missing at random (i.e., they are indicative of very low or very high values), which poses a unique challenge for data analyses. When data are missing due to levels being too low to detect, researchers may consider imputing either 0 or a very small value for missing data (i.e., the lower limit of detection, half the lower limit of detection, or the lowest standard) (Riis et al., 2020). This approach uses all available data and is a common strategy when examining salivary markers of inflammation (e.g., Byrne et al., 2013; Newton et al., 2017; Slavish et al., 2019; Szabo et al., 2019). One recent study compared eight strategies for handling salivary CRP data missing due to high values, including listwise deletion, Winsorization, and multiple imputation. Winsorization emerged as a promising approach for high out of range samples (Landau et al., 2019).

Directions for Future Research

Future studies also should carefully consider the short- and long-term stability of salivary inflammatory markers. Among adolescent girls, the mean correlation between salivary markers of inflammation (e.g., TNF-α, IL-1β, IL-6, CRP, IL-8) assessed two hours apart was $r = 0.67$, but the mean correlation between these same markers assessed 18 months apart was $r = 0.18$ (although this correlation increased to $r = 0.27$ when averaging two samples within each session) (Shields et al., 2019). Other studies have similarly shown salivary CRP and IL-6 are moderately stable across two days ($r = 0.55$ to 0.96), although CRP appears to be more stable ($r = .77$ to .96) than IL-6 ($r = .55$ to .77) (Izawa et al., 2013a) and that the correlation of CRP reduces across time (baseline with 1 year, $r = .61$; with year 2, $r = .46$; Out et al., 2012). This work highlights that many salivary markers of inflammation are likely relatively stable across shorter periods of time (hours to days) but may fluctuate drastically over longer periods of time (months to years). Future studies may capitalize on this long-term instability by examining predictors of within-person changes in salivary markers across time.

There is also a critical need for studies examining correspondence of inflammatory responses in both saliva and blood (extant literature summarized in Table 1). It would also be helpful to examine the simultaneous predictive validity of blood- and salivary-based inflammatory biomarkers for disease states. The current state of the field is also limited by a lack of longitudinal or prospective study designs, which would help better infer causality.

Together, these types of studies may help inform when salivary markers can serve as a replacement for blood-based markers in psychoneuroimmunology research.

Summary and Conclusions

Researchers often weigh study design considerations against limited time, financial resources, and the ability to recruit the desired study population. Salivary markers of inflammation represent a promising low burden option for both participants and researchers. Guided by current research in this area, this paper serves as comprehensive and practical summary for researchers interested in incorporating these measures into their study protocols. Additional work is needed to elucidate gold standards for study design, measurement, and analysis, but salivary markers of inflammation may be a useful tool for understanding oral and peripheral inflammation dynamics non-invasively.

Acknowledgements

This material is the result of work partially supported by a Career Development Award from the Department of Veterans Affairs Rehabilitation Research & Development Service (IK1-RX003122) awarded to YZS and the use of facilities at the VISN 17 Center of Excellence for Research on Returning War Veterans. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs, United States government, or academic affiliates, nor were these sources involved in study design, data collection, or interpretation of findings.

References

Arellano-Garcia ME, Hu S, Wang J, Henson B, Zhou H, Chia D, & Wong DT (2008). Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. Oral Diseases, 14(8), 705–712. doi:10.1111/j.1601-0825.2008.01488.x [PubMed: 19193200]

Auer BJ, Calvi JL, Jordan NM, Schrader D, & Byrd-Craven J (2018). Communication and social interaction anxiety enhance interleukin-1 beta and cortisol reactivity during high-stakes public speaking. Psychoneuroendocrinology, 94, 83–90. doi:10.1016/j.psyneuen.2018.05.011 [PubMed: 29775877]

Azar R, & Richard A (2011). Elevated salivary C-reactive protein levels are associated with active and passive smoking in healthy youth: A pilot study. Journal of Inflammation, 8(1), 37. doi:10.1186/1476-9255-8-37 [PubMed: 22152006]

Bellagambi FG, Lomonaco T, Salvo P, Vivaldi F, Hangouët M, Ghimenti S, Biagini D, Di Francesco F, Fuoco R, & Errachid A (2020). Saliva sampling: Methods and devices. An overview. In TrAC - Trends in Analytical Chemistry. doi:10.1016/j.trac.2019.115781

Bosch JA (2014). The use of saliva markers in psychobiology: Mechanisms and methods. Monographs in Oral Science: Secretion and Functions, 94, 99–108. doi:10.1159/000358864

Buhlin K, Gustafsson A, Andersson K, Håkansson J, & Klinge B (2002). Validity and limitations of self-reported periodontal health. Community Dentistry and Oral Epidemiology, 30(6), 431–437. doi:10.1034/j.1600-0528.2002.00014.x [PubMed: 12453114]

Buzgoova K, Balagova L, Marko M, Kapsdorfer D, Riecansky I, & Jezova D (2020). Higher perceived stress is associated with lower cortisol concentrations but higher salivary interleukin-1beta in socially evaluated cold pressor test. Stress, 23(3), 248–255. doi:10.1080/10253890.2019.1660872 [PubMed: 31466500]

Byrne ML, O'Brien-Simpson NM, Reynolds EC, Walsh KA, Laughton K, Waloszek JM, Woods MJ, Trinder J, & Allen NB (2013). Acute phase protein and cytokine levels in serum and saliva: A comparison of detectable levels and correlations in a depressed and healthy adolescent sample. Brain, Behavior, and Immunity, 34, 164–175. doi:10.1016/j.bbi.2013.08.010

Calsina G, Ramón JM, & Echeverría JJ (2002). Effects of smoking on periodontal tissues. Journal of Clinical Periodontology, 29(8), 771–776. doi:10.1034/j.1600-051X.2002.290815.x [PubMed: 12390575]

Caris AV, da Silva ET, dos Santos SA, Tufik S, & dos Santos RVT (2017). Effects of carbohydrate and glutamine supplementation on oral mucosa immunity after strenuous exercise at high altitude: A double-blind randomized trial. Nutrients, 9(7), 692. doi:10.3390/nu9070692

Centers for Disease Control and Prevention. (2019). Oral Health Surveillance Report: Trends in Dental Caries and Sealants, Tooth Retention, and Edentulism, United States, 1999–2004 to 2011–2016.

Cermakian N, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, & Mazzoccoli G (2013). Crosstalk between the circadian clock circuitry and the immune system. Chronobiology International, 30(7), 870–888. doi:10.3109/07420528.2013.782315 [PubMed: 23697902]

Chauhan A, Yadav SS, Dwivedi P, Lal N, Usman K, & Khattri S (2016). Correlation of Serum and Salivary Cytokines Level With Clinical Parameters in Metabolic Syndrome With Periodontitis. Journal of Clinical Laboratory Analysis, 30(5), 649–655. doi:10.1002/jcla.21917 [PubMed: 26899213]

Coogan AN, & Wyse CA (2008). Neuroimmunology of the circadian clock. Brain Research, 104–112. doi:10.1016/j.brainres.2008.07.087

Cruz-Almeida Y, Aguirre M, Sorenson H, Tighe P, Wallet SM, & Riley JL (2017). Age differences in salivary markers of inflammation in response to experimental pain: Does venipuncture matter? Journal of Pain Research, 10, 2365. doi:10.2147/JPR.S138460 [PubMed: 29042812]

Cuesta M, Boudreau P, Dubeau-Laramée G, Cermakian N, & Boivin DB (2016). Simulated night shift disrupts circadian rhythms of immune functions in humans. The Journal of Immunology, 196(6), 2466–2475. doi:10.4049/jimmunol.1502422 [PubMed: 26873990]

Cullen T, Thomas AW, Webb R, & Hughes MG (2015). The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. Cytokine. doi:10.1016/j.cyto.2014.10.011

Dan H, Liu W, Wang J, Wang Z, Wu R, Chen Q, Zeng X, & Zhou Y (2011). Elevated IL-10 concentrations in serum and saliva from patients with oral lichen planus. Quintessence International, 42(2), 157–163. [PubMed: 21359250]

de Jager W, Bourcier K, Rijkers G, Prakken B, & Seyfert-Margolis V (2009). Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC Immunology, 10(52). doi:10.1186/1471-2172-10-52

De Oliveira DMP, Hartley-Tassell L, Everest-Dass A, Day CJ, Dabbs RA, Ve T, Kobe B, Nizet V, Packer NH, Walker MJ, Jennings MP, & Sanderson-Smith ML (2017). Blood group antigen recognition via the group a streptococcal m protein mediates host colonization. MBio, 8(1). doi:10.1128/mBio.02237-16

Desai GS, & Mathews ST (2014). Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World Journal of Diabetes, 5(6), 730–738. doi:10.4239/wjd.v5.i6.730 [PubMed: 25512775]

Dillon MC, Opris DC, Kopanczyk R, Lickliter J, Cornwell HN, Bridges EG, Nazar AM, & Bridges KG (2010). Detection of homocysteine and C-reactive protein in the saliva of healthy adults: Comparison with blood levels. Biomarker Insights, 5, BMI–S5305. doi:10.4137/bmi.s5305

Dogra S, Wolf M, Jeffrey MP, Foley RCA, Logan-Sprenger H, Jones-Taggart H, & Green-Johnson JM (2019). Disrupting prolonged sitting reduces IL-8 and lower leg swell in active young adults. BMC Sports Science, Medicine and Rehabilitation, 11(1), 23. doi:10.1186/s13102-019-0138-4

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, & Lanctôt KL (2010). A meta-analysis of cytokines in major depression. Biological Psychiatry, 67(5), 446–457. doi:10.1016/j.biopsych.2009.09.033 [PubMed: 20015486]

DuPont NC, Wang K, Wadhwa PD, Culhane JF, & Nelson EL (2005). Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: Determinations of a panel of nine cytokines in clinical sample culture supernatants. Journal of Reproductive Immunology, 66(2), 175–191. doi:10.1016/j.jri.2005.03.005 [PubMed: 16029895]

Ebersole JL, Kryscio RJ, Campbell C, Kinane DF, McDevitt J, Christodoulides N, Floriano PN, & Miller CS (2017). Salivary and serum adiponectin and C-reactive protein levels in acute

myocardial infarction related to body mass index and oral health. Journal of Periodontal Research, 52(3), 419–427. doi:10.1111/jre.12406 [PubMed: 27549083]

Eke PI, Thornton-Evans GO, Wei L, Borgnakke WS, Dye BA, & Genco RJ (2018). Periodontitis in US Adults: National Health and Nutrition Examination Survey 2009–2014. Journal of the American Dental Association, 149(7), 576–588. doi:10.1016/j.adaj.2018.04.023 [PubMed: 29957185]

Engeland CG, Bosch JA, & Rohleder N (2019). Salivary biomarkers in psychoneuroimmunology. Current Opinion in Behavioral Sciences, 28, 58–65. doi:10.1016/j.cobeha.2019.01.007 [PubMed: 32215283]

Fernandez-Botran R, Miller JJ, Burns VE, & Newton TL (2011). Correlations among inflammatory markers in plasma, saliva and oral mucosal transudate in post-menopausal women with past intimate partner violence. Brain, Behavior, and Immunity, 25(2), 314–321. doi:10.1016/j.bbi.2010.09.023

Filaire E, Larue J, Portier H, Abed A, Graziella PD, Teixeira A, & Anne P (2011). Lecturing to 200 students and its effects on cytokine concentration and salivary markers of adrenal activation. Stress and Health, 27(2), e25–e35. doi:10.1002/smi.1332 [PubMed: 27486621]

Gan SD, & Patel KR (2013). Enzyme immunoassay and enzyme-linked immunosorbent assay. Journal of Investigative Dermatology, 133(9), e12. doi:10.1038/jid.2013.287

Goetz SM, & Lucas T (2020). C-reactive protein in saliva and dried blood spot as markers of stress reactivity in healthy African-Americans. Biomarkers in Medicine, 14(5), 371–380. doi:10.2217/bmm-2019-0391 LK [PubMed: 32253920]

Gohel V, Jones JA, & Wehler CJ (2018). Salivary biomarkers and cardiovascular disease: A systematic review. Clinical Chemistry and Laboratory Medicine (CCLM), 56(9), 1432–1442. doi:10.1515/cclm-2017-1018 [PubMed: 29630504]

Granger DA, Johnson SB, Szanton SL, Out D, & Schumann LL (2012). Incorporating salivary biomarkers into nursing research: An overview and review of best practices. Biological Research for Nursing, 14(4), 347–356. doi:10.1177/1099800412443892 [PubMed: 22593229]

Granger DA, Kivlighan KT, Fortunato C, Harmon AG, Hibel LC, Schwartz EB, & Whembolua GL (2007). Integration of salivary biomarkers into developmental and behaviorally-oriented research: Problems and solutions for collecting specimens. Physiology and Behavior, 92(4), 583–590. doi:10.1016/j.physbeh.2007.05.004 [PubMed: 17572453]

Granger DA, & Taylor MK (Eds.). (2020). Salivary Bioscience: Foundations of Interdisciplinary Saliva Research and Applications. Springer International Publishing. doi:10.1007/978-3-030-35784-9

Gröschl M, Köhler H, Topf HG, Rupprecht T, & Rauh M (2008). Evaluation of saliva collection devices for the analysis of steroids, peptides and therapeutic drugs. Journal of Pharmaceutical and Biomedical Analysis, 47(3), 478–486. doi:10.1016/j.jpba.2008.01.033 [PubMed: 18325706]

Guggenheimer J, & Moore PA (2003). Xerostomia: Etiology, recognition and treatment. Journal of the American Dental Association, 134(1), 61–69. doi:10.14219/jada.archive.2003.0018 [PubMed: 12555958]

Helmi ZR, Sabri RA, & Hameed BH (2016). Assessment of oral health status, leptin, and inflammatory markers in serum and saliva of patients with polycystic ovarian syndrome in reference to metabolic syndrome. Mustansiriya Medical Journal, 15(1), 11–19.

Hirtz C, Vialaret J, Nowak N, Gabelle A, Deville De Périère D, & Lehmann S (2016). Absolute quantification of 35 plasma biomarkers in human saliva using targeted MS. Bioanalysis, 8(1), 43–53. doi:10.4155/bio.15.228 [PubMed: 26647646]

Hosseinzadeh A, Thompson PR, Segal BH, & Urban CF (2016). Nicotine induces neutrophil extracellular traps. Journal of Leukocyte Biology, 100(5), 1105–1112. doi:10.1189/jlb.3ab0815-379rr [PubMed: 27312847]

Iyengar A, Paulus JK, Gerlanc DJ, & Maron JL (2014). Detection and potential utility of C-reactive protein in saliva of neonates. Frontiers in Pediatrics, 2, 131. doi:10.3389/fped.2014.00131 [PubMed: 25485262]

Izawa S, Miki K, Liu X, & Ogawa N (2013). The diurnal patterns of salivary interleukin-6 and C-reactive protein in healthy young adults. Brain, Behavior, and Immunity, 27, 38–41. doi:10.1016/j.bbi.2012.07.001

Izawa S, Sugaya N, Kimura K, Ogawa N, Yamada KC, Shirotsuki K, Mikami I, Hirata K, Nagano Y, & Nomura S (2013b). An increase in salivary interleukin-6 level following acute psychosocial stress and its biological correlates in healthy young adults. Biological Psychology, 94(2), 249–254. doi:10.1016/j.biopsycho.2013.06.006 [PubMed: 23831278]

Jabber WF, Zaidan TF, Gorial FI, & Al-Naaimi AS (2015). Salivary interleukin 6 is a valid biomarker for diagnosis of osteoporosis in postmenopausal women. Chemistry and Materials Research, 7(7), 65–69.

Jacobs R, Tshehla E, Malherbe S, Kriel M, Loxton AG, Stanley K, van der Spuy G, Walzl G, & Chegou NN (2016). Host biomarkers detected in saliva show promise as markers for the diagnosis of pulmonary tuberculosis disease and monitoring of the response to tuberculosis treatment. Cytokine, 81, 50–56. doi:10.1016/j.cyto.2016.02.004 [PubMed: 26878648]

Jaedicke KM, Preshaw PM, & Taylor JJ (2016). Salivary cytokines as biomarkers of periodontal diseases. Periodontology 2000, 164–183. doi:10.1111/prd.12117

Janket SJ, Baird AE, Chuang SK, & Jones JA (2003). Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. doi:10.1067/moe.2003.107

Kamodyová N, Baňasová L, Janšáková K, Koborová I, Tóthová ubomíra, Stanko P, & Celec P (2015). Blood contamination in saliva: Impact on the measurement of salivary oxidative stress markers. Disease Markers. doi:10.1155/2015/479251

Kendall-Tackett K (Ed.). (2010). The psychoneuroimmunology of chronic disease: Exploring the links between inflammation, stress, and illness. American Psychological Association. doi:10.1037/12065-000

Kimura K, Izawa S, Sugaya N, Ogawa N, Yamada KC, Shirotsuki K, Mikami I, Hirata K, Nagano Y, & Hasegawa T (2013). The biological effects of acute psychosocial stress on delay discounting. Psychoneuroendocrinology, 38(10), 2300–2308. doi:10.1016/j.psyneuen.2013.04.019 [PubMed: 23768971]

Kirschbaum C, Pirke KM, & Hellhammer DH (1993). The “Trier social stress test” - A tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology. doi:10.1159/000119004

Kivlighan KT, Granger DA, Schwartz EB, Nelson V, Curran M, & Shirtcliff EA (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. Hormones and Behavior, 46(1), 39–46. doi:10.1016/j.yhbeh.2004.01.006 [PubMed: 15215040]

Kopanczyk R, Opris DC, Lickliter J, Bridges EG, Nazar AM, & Bridges KG (2010). C-reactive protein levels in blood and saliva show no correlation in young, healthy adults. The FASEB Journal. doi:10.1096/fasebj.24.1_supplement.lb409

La Fratta I, Tatangelo R, Campagna G, Rizzuto A, Franceschelli S, Ferrone A, Patruno A, Speranza L, De Lutiis MA, Felaco M, Grilli A, & Pesce M (2018). The plasmatic and salivary levels of IL-1β, IL-18 and IL-6 are associated to emotional difference during stress in young male. Scientific Reports, 8(1), 1–10. doi:10.1038/s41598-018-21474-y [PubMed: 29311619]

LaMonte MJ, Hovey KM, Millen AE, Genco RJ, & Wactawski-Wende J (2014). Accuracy of self-reported periodontal disease in the Women’s Health Initiative Observational Study. Journal of Periodontology, 85(8), 1006–1018. doi:10.1902/jop.2013.130488 [PubMed: 24354649]

Landau ER, Trinder J, Simmons JG, Raniti M, Blake M, Waloszek JM, Blake L, Schwartz O, Murray G, Allen NB, & Byrne ML (2019). Salivary C-reactive protein among at-risk adolescents: A methods investigation of out of range immunoassay data. Psychoneuroendocrinology, 99, 104–111. doi:10.1016/j.psyneuen.2018.08.035 [PubMed: 30219639]

Laurent HK, Lucas T, Pierce J, Goetz S, & Granger DA (2016). Coordination of cortisol response to social evaluative threat with autonomic and inflammatory responses is moderated by stress appraisals and affect. Biological Psychology, 118, 17–24. doi:10.1016/j.biopsycho.2016.04.066 [PubMed: 27155141]

Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, & Chang KW (2018). Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. International Journal of Oral and Maxillofacial Surgery. doi:10.1016/j.ijom.2017.09.016

Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, & Kuchel GA (2008). ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. Journals of Gerontology - Series A Biological Sciences and Medical Sciences, 63(8), 879–884. doi:10.1093/gerona/63.8.879

Matevosyan NR (2010). Oral health of adults with serious mental illnesses: A review. Community Mental Health Journal, 46(6), 553–562. doi:10.1007/s10597-009-9280-x [PubMed: 20039129]

McEvoy JW, Nasir K, Defilippis AP, Lima JAC, Bluemke DA, Hundley WG, Barr RG, Budoff MJ, Szklo M, Navas-Acien A, Polak JF, Blumenthal RS, Post WS, & Blaha MJ (2015). Relationship of cigarette smoking with inflammation and subclinical vascular disease: The multi-ethnic study of atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology, 35(4), 1002–1010. doi:10.1161/ATVBAHA.114.304960

Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, & Mullington JM (2001). Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. Clinical Chemistry, 47(3), 426–430. doi:10.1093/clinchem/47.3.426 [PubMed: 11238292]

Michopoulos V, Powers A, Gillespie CF, Ressler KJ, & Jovanovic T (2017). Inflammation in fear-and anxiety-based disorders: PTSD, GAD, and beyond. Neuropsychopharmacology, 42(1), 254–270. doi:10.1038/npp.2016.146 [PubMed: 27510423]

Miller CS, King CP, Langub MC, Kryscio RJ, & Thomas MV (2006). Salivary biomarkers of existing periodontal disease: A cross-sectional study. Journal of the American Dental Association, 137, 322–329. doi:10.14219/jada.archive.2006.0181 [PubMed: 16570465]

Miller GE, & Blackwell E (2006). Turning up the heat: Inflammation as a mechanism linking chronic stress, depression, and heart disease. Current Directions in Psychological Science, 15(6), 269–272. doi:10.1111/j.1467-8721.2006.00450.x

Minetto MA, Gazzoni M, Lanfranco F, Baldi M, Saba L, Pedrola R, Komi PV, & Rainoldi A (2007). Influence of the sample collection method on salivary interleukin-6 levels in resting and post-exercise conditions. European Journal of Applied Physiology, 101(2), 249–256. doi:10.1007/s00421-007-0484-x [PubMed: 17569075]

Minetto M, Rainoldi A, Gazzoni M, Terzolo M, Borrione P, Termine A, Saba L, Dovio A, Angeli A, & Paccotti P (2005). Differential responses of serum and salivary interleukin-6 to acute strenuous exercise. European Journal of Applied Physiology, 93(5–6), 679–686. doi:10.1007/s00421-004-1241-z [PubMed: 15778897]

Mohamed R, Campbell J-L, Cooper-White J, Dimeski G, & Punyadeera C (2012). The impact of saliva collection and processing methods on CRP, IgE, and Myoglobin immunoassays. Clinical and Translational Medicine, 1(1), 19. doi:10.1186/2001-1326-1-19 [PubMed: 23369566]

Nam Y, Kim YY, Chang JY, & Kho HS (2019). Salivary biomarkers of inflammation and oxidative stress in healthy adults. Archives of Oral Biology, 97, 215–222. doi:10.1016/j.archoralbio.2018.10.026 [PubMed: 30399508]

Närhi TO, Meurman JH, & Ainamo A (1999). Xerostomia and hyposalivation: Causes, consequences and treatment in the elderly. Drugs and Aging, 15(2), 103–116. doi:10.2165/00002512-199915020-00004 [PubMed: 10495070]

Newton TL, Fernandez-Botran R, Lyle KB, Szabo YZ, Miller JJ, & Warnecke AJ (2017). Salivary cytokine response in the aftermath of stress: An emotion regulation perspective. Emotion, 17(6), 1007. doi:10.1037/emo0000156 [PubMed: 28287751]

Newton TL, Fernandez-Botran R, Miller JJ, & Burns VE (2014). Interleukin-6 and soluble interleukin-6 receptor levels in posttraumatic stress disorder: Associations with lifetime diagnostic status and psychological context. Biological Psychology, 99, 150–159. doi:10.1016/j.biopsycho.2014.03.009 [PubMed: 24695006]

O'Connor MF, Bower JE, Cho HJ, Creswell JD, Dimitrov S, Hamby ME, Hoyt MA, Martin JL, Robles TF, Sloan EK, Thomas KMS, & Irwin MR (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. Brain, Behavior, and Immunity. doi:10.1016/j.bbi.2009.04.005

Ouellet-Morin I, Danese A, Williams B, & Arseneault L (2011). Validation of a high-sensitivity assay for C-reactive protein in human saliva. Brain, Behavior, and Immunity, 25(4), 640–646. doi:10.1016/j.bbi.2010.12.020

Out D, Hall RJ, Granger DA, Page GG, & Woods SJ (2012). Assessing salivary C-reactive protein: Longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. Brain, Behavior, and Immunity, 26, 543–551. doi:10.1016/j.bbi.2012.01.019

Padilla GA, Calvi JL, Taylor MK, & Granger DA (2020). Saliva collection, handling, transport, and storage: Special considerations and best practices for interdisciplinary salivary bioscience research. In Salivary Bioscience (pp. 21–47). doi:10.1007/978-3-030-35784-9_3

Pappa E, Kousvelari E, & Vastardis H (2019). Saliva in the “Omics” era: A promising tool in paediatrics. In Oral Diseases (pp. 16–25). doi:10.1111/odi.12886 [PubMed: 29750386]

Pedersen AM, Bardow A, Jensen SB, & Nauntofte B (2002). Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral Diseases, 8(3), 117–129. doi:10.1034/j.1601-0825.2002.02851.x [PubMed: 12108756]

Quinn ME, Stanton CH, Slavich GM, & Joormann J (2020). Executive control, cytokine reactivity to social stress, and depressive symptoms: Testing the social signal transduction theory of depression. Stress, 23(1), 60–68. doi:10.1080/10253890.2019.1641079 [PubMed: 31364435]

Reed RG, O’Connor MF, Pace TWW, Raison CL, & Butler EA (2017). Dyadic coping and salivary interleukin-6 responses to interpersonal stress. Journal of Family Psychology, 31(3), 367. doi:10.1037/fam0000249 [PubMed: 27668934]

Reinhardt ÉL, Fernandes PACM, Markus RP, & Fischer FM (2016). Short sleep duration increases salivary IL-6 production. Chronobiology International, 33(6), 780–782. doi:10.3109/07420528.2016.1167710 [PubMed: 27070477]

Reinhardt ÉL, Fernandes PACM, Markus RP, & Fischer FM (2019). Night work effects on salivary cytokines TNF, IL-1β and IL-6. Chronobiology International, 36(1), 11–26. doi:10.1080/07420528.2018.1515771 [PubMed: 30230913]

Riis JL, Ahmadi H, Hamilton KR, Hand T, & Granger DA (2020). Best Practice Recommendations for the Measurement and Interpretation of Salivary Proinflammatory Cytokines in Biobehavioral Research. Brain, Behavior, and Immunity. doi:10.1016/j.bbi.2020.09.009

Riis JL, Byrne ML, Hernández LM, & Robles TF (2020). Salivary Bioscience, Immunity, and Inflammation. In Salivary Bioscience (pp. 177–213). Springer.

Riis Jenna L., Out D, Dorn LD, Beal SJ, Denson LA, Pabst S, Jaedicke K, & Granger DA (2014). Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage. Developmental Psychobiology, 56(4), 797–811. doi:10.1002/dev.21149 [PubMed: 23868603]

Salimetrics. (2020a). Collecting and Handling Saliva Samples for Cytokine Analysis.

Salimetrics. (2020b). Saliva Collection Handbook.

Saraev GB, Mironova ES, Linkova NS, Bunin VA, Paltsev MA, & Kvetnoy IM (2019). Investigation of signal molecules in saliva: prospects of application for diagnostics of myocardial infarction and the aging rate of different age people. Advances in Gerontology = Uspekhi Gerontologii, 32(3), 364–369. [PubMed: 31512422]

Shields GS, Slavich GM, Perlman G, Klein DN, & Kotov R (2019). The short-term reliability and long-term stability of salivary immune markers. Brain, Behavior, and Immunity, 81, 650–654. doi:10.1016/j.bbi.2019.06.007

Ship JA, Pillemer SR, & Baum BJ (2002). Xerostomia and the geriatric patient. Journal of the American Geriatrics Society, 535–543. doi:10.1046/j.1532-5415.2002.50123.x [PubMed: 11943053]

Shirtcliff EA, Granger DA, Schwartz E, & Curran MJ (2001). Use of salivary biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology, 26(2), 165–173. doi:10.1016/S0306-4530(00)00042-1 [PubMed: 11087962]

Silvestre-Rangil J, Bagán L, Silvestre FJ, Martinez-Herrera M, & Bagán J (2017). Periodontal, salivary and IL-6 status in rheumatoid arthritis patients: A cross-sectional study. Medicina Oral, Patologia Oral y Cirugia Bucal, 22(5), e595–e600. doi:doi:10.4317/medoral.21937

Slavish DC, Jones DR, Smyth JM, Engeland CG, Song S, McCormick NM, & Graham-Engeland JE (2019). Positive and negative affect and salivary markers of inflammation among young adults. International Journal of Behavioral Medicine, 27(3), 282–293.

Slavish Danica C., Graham-Engeland JE, Smyth JM, & Engeland CG (2015). Salivary markers of inflammation in response to acute stress. In Brain, Behavior, and Immunity (pp. 253–269). doi:10.1016/j.bbi.2014.08.008

Slavish Danica C., & Szabo YZ (2019). The effect of acute stress on salivary markers of inflammation: A systematic review protocol. Systematic Reviews, 108(8), 1–8. doi:10.1186/s13643-019-1026-4

Subbarao K, Nattuthurai G, Sundararajan S, Sujith I, Joseph J, & Syedshah Y (2019). Gingival crevicular fluid: An overview. In Journal of Pharmacy and Bioallied Sciences (pp. S135–S139). doi:10.4103/JPBS.JPBS_56_19 [PubMed: 31198325]

Szabo YZ, Slavish DC, & Graham-Engeland JE (2020). The effect of acute stress on salivary markers of inflammation: A systematic review and meta-analysis. Brain, Behavior, and Immunity, 88, 887–900. doi:10.1016/j.bbi.2020.04.078

Szabo YZ, Fernandez-Botran R, & Newton TL (2019). Cumulative trauma, emotion reactivity and salivary cytokine levels following acute stress in healthy women. Anxiety, Stress and Coping, 32(1), 82–94. doi:10.1080/10615806.2018.1524377

Szabo YZ, Newton TL, Miller JJ, Lyle KB, & Fernandez-Botran R (2016). Acute stress induces increases in salivary IL-10 levels. Stress, 19(5), 499–505. doi:10.1080/10253890.2016.1206885 [PubMed: 27353112]

Tell D, Mathews HL, Burr RL, & Janusek LW (2018). During stress, heart rate variability moderates the impact of childhood adversity in women with breast cancer. Stress, 21(2), 179–187. doi:10.1080/10253890.2018.1424132 [PubMed: 29385886]

Tennent GA, Brennan SO, Stangou AJ, O'Grady J, Hawkins PN, & Pepys MB (2007). Human plasma fibrinogen is synthesized in the liver. Blood, 109(5), 1971–1974. doi:10.1182/blood-2006-08-040956 [PubMed: 17082318]

Usui T, Yoshikawa T, Ueda SY, Katsura Y, Orita K, & Fujimoto S (2011). Effects of acute prolonged strenuous exercise on the salivary stress markers and inflammatory cytokines. Japanese Journal of Physical Fitness and Sports Medicine, 60(3), 295–304. doi:10.7600/jspfsm.60.295

Van Der Vaart H, Postma DS, Timens W, & Ten Hacken NHT (2004). Acute effects of cigarette smoke on inflammation and oxidative stress: A review. Thorax, 59(8), 713–721. doi:10.1136/thx.2003.012468 [PubMed: 15282395]

Vgontzas AN, Bixler EO, Lin HM, Prolo P, Trakada G, & Chrousos GP (2005). IL-6 and its circadian secretion in humans. Neuroimmunomodulation, 12(3), 131–140. doi:10.1159/000084844 [PubMed: 15905620]

Vincent FB, Nim HT, Lee JPW, Morand EF, & Harris J (2019). Effect of storage duration on cytokine stability in human serum and plasma. Cytokine, 113, 453–457. doi:10.1016/j.cyto.2018.06.009 [PubMed: 29909979]

Wang Z, Mandel H, Levingston CA, & Young MRI (2016). An exploratory approach demonstrating immune skewing and a loss of coordination among cytokines in plasma and saliva of Veterans with combat-related PTSD. Human Immunology, 77(8), 652–657. doi:10.1016/j.humimm.2016.05.018 [PubMed: 27216157]

Whembolua GLS, Granger DA, Singer S, Kivlighan KT, & Marguin JA (2006). Bacteria in the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. Hormones and Behavior, 49(4), 478–483. doi:10.1016/j.yhbeh.2005.10.005 [PubMed: 16309679]

Williamson S, Munro C, Pickler R, Grap MJ, & Elswick RK (2012). Comparison of biomarkers in blood and saliva in healthy adults. Nursing Research and Practice, e246178. doi:10.1155/2012/246178

Wilson SJ, Woody A, & Kiecolt-Glaser JK (2018). Inflammation as a biomarker method in lifespan developmental methodology. In Oxford Research Encyclopedia of Psychology. doi:10.1093/acrefore/9780190236557.013.355

Yeung D, Ciotti S, Purushothama S, Gharakhani E, Kuesters G, Schlain B, & Mikulskis A (2016). Evaluation of highly sensitive immunoassay technologies for quantitative measurements of sub-

pg/mL levels of cytokines in human serum. Journal of Immunological Methods, 437, 53–63. doi:10.1016/j.jim.2016.08.003 [PubMed: 27555238]

Table 1

Studies Focused on Correlations between Measures of Inflammation in Saliva and Blood

Study and Sample	Sample Collection Methods	CRP	IL-1β	IL-6	TNF-α	IL-10	IFN-γ
Byrne et al., 2013 – 18 healthy and 17 depressed adolescents (22.2% male) (n =17–32; non parametric analyses reported)	Serum passive drool samples taken between 9 am and noon	Non-detectable samples excluded: medium correlation (r = 0.42, p = 0.02), high CRP subsample (r = 0.60, p = 0.01). Non-detectable samples included as 0, medium correlation (r = 0.39, p = 0.02).			Non-detectable samples were included as 0, medium correlation (r = 0.32, p = 0.06)	Non-detectable samples were included as 0, medium correlation (r = 0.42, p = 0.02)	
Cullen et al., 2015– 10 healthy young adults (50% men, 50% women)	Plasma passive drool time of day not reported			Medium correlations: at rest (r = −.38, p = .85); post- exercise (r = .49, p = .12)			
Dan et al., 2011 – 79 Chinese adults with oral lichen planus; 41 healthy controls (47.5% male)	Serum spitting samples taken between 8 and 10 am					Large correlation in clinical sample (r = 0.59, p < .001), not reported for controls	Large correlation in clinical sample, (r = 0.78, p < .001), not reported for controls
Dillon et al., 2010 – 55 healthy medical school students (full sample of 69 was 48% female)	Plasma passive drool time not specified	No correlation (R^2=0.001), did not differ if flow rate adjusted. Large correlation for 5 students with high CRP (r = 0.71, p = 0.12)					
Ebersole et al., 2017 – 203 adults (29.3% female), some with acute myocardial infarction (MI) and healthy controls	Serum saliva collection method not reported time of day not reported	Correlation in control subjects (p < 0.008), but not MI subjects.					
Fernandez-Botran et al., 2011– 67 physically healthy midlife women; mixed mental health status	Plasma saliva sampler (cellulose pad) samples taken between 8 am and 1 pm			Small to medium correlation (visit 1: r = .29, p = .02; visit 2: r = .10, p = .41)			
Iyengar et al., 2014– 35 neonates (gender not reported)	Serum Syringe saliva collection time of day varied	Large correlation (raw: r = 0.68, p < 0.001; protein adjusted, r = 0.60, p < 0.001)					
La Fratta et al., 2018– 61 healthy young men	Plasma Salivette samples were taken 12 pm to 3 pm	Medium correlations on rest day (r =.40), large correlation pre- (r = .65) and post exam (r = .63, all p < .01)					

Study and Sample	Sample Collection Methods	CRP	IL-1β	IL-6	TNF-α	IL-10	IFN-γ
Lee et al., 2018, 41 adults with oral cancer and 24 adults with non-oral cancer (86.2% male)	Plasma Saliva collection method not specified time not specified		Weak correlation (data not shown)	Weak correlation (data not shown)	Weak correlation (data not shown)	Weak correlation (data not shown)	Weak correlation (data not shown)
Out et al., 2012– 107 adults (100% female) with intimate partner abuse histories - mixed health	Plasma; passive drool; blood drawn between 6 and 9:30 pm, saliva collected am & pm then averaged.	Medium to large correlations: baseline, (r = .53, p < .01), year 1 (r = .38, p < .01), year 2 (r = .49, p < .01)					
Ouellet-Morin et al., 2011– 61 healthy adults (70.5% female); n = 10 with high CRP removed for analysis	Serum passive drool sessions took place between 10 am and 2 pm	Large correlation (r = .72, p < .001), adjusted for sex and age (each r = .73, p < .001)					
Nam et al., 2019– 27 healthy young men	Serum or plasma not indicated spitting; samples taken between 8:00 and 9:30 am	Small correlation (r = −.17, p = 0.31)	No correlation (r =.09, p = 0.58)	Medium correlation (r = .36, p = 0.03)	Small correlation (r = −.15, p = 0.38)		
Riis et al., 2014– 107–113 adolescents (100% female) missing data imputed at 0	Serum Salivette samples taken between 11:30 am and 12:30 pm		Small correlation (r = .11, *ns*), controlling for age.	No correlation (r = .07, *ns*), controlling for age.	No correlation (r = −.08, ns), controlling for age.	Not detectable	Not detectable
Wang et al., 2016 13 Veterans (92.3% male; 7 with PTSD, 6 without)	Plasma passive drool samples taken midafternoon			PTSD: Large correlation (r =.71, p < 0.05); no correlation for controls (data not shown)	No significant correlation (data not shown)	PTSD: Large correlation (r =.86, p < 0.01); no correlation for controls (data not shown)	
Williamson et al., 2012– 50 healthy adults (52% men)	Plasma Passive drool and filter paper time of day not reported		No to small correlation: filter paper (r = .01, *ns*), passive drool (r = .12, *ns*)	Small to medium correlation: filter paper (r = .25, *ns*), passive drool (r = .31, p < .05)	Small correlation: filter paper (r = .19, *ns*), passive drool (r = .17; *ns*)	Small correlation filter paper (r = .21, *ns*), passive drool (r = .28; *ns*)	Small to medium correlation: filter paper (r = .13, *ns*), passive drool (r = .34, p < .05)

Note. CRP = C-reative protein, IL = interleukin, TNF = tumor necrosis factor, IFN = interferon; Rather than statistical significance, the above table uses effect sizes of small = .10, medium = .30 and large = .50, ns = not significant

Table 2.

Factors to Measure, Analyze, and Report when Collecting Salivary Markers of Inflammation

What to assess ahead of time or recruit for	What to restrict or measure before sample collection	What to record while sampling	What to consider for analyses	What to report in manuscript
• Medical conditions, particularly those that may influence oral health or saliva production • Oral health status (e.g., presence of dental cavities, swollen or bleeding gums, recent dental procedures) * • Oral hygiene (e.g., teeth brushing and flossing habits, number of teeth) • Medication use, including contraceptive use and anti-inflammatory medications • Menstrual cycle status • Smoking status • Other drug use • Demographic information (age, sex/gender, race/ethnicity) • BMI	• Caffeine use • Alcohol use • Smoking • Other drug use • Food/drinking •Exercise • Medication use • Fever and other physical symptoms that may indicate acute infection (runny nose, sore throat, nausea, rash/swelling) • Acute injury	• Time of sample collection • How long it took to collect sample (for salivary flow rate calculation) • Visible presence of blood in sample • Volume or weight of sample	• Missing data/non-detectable levels (exclude vs. impute 0 vs. impute LLD) • Data skew / transformations • Relevant covariates or possibly confounding variables (e.g., BMI, medical conditions, medication use) • Salivary flow rate adjustment	• Amount of missing data/non-detectable levels and imputation methods • Salivary flow rate • Intra- and inter-assay coefficients of variation • Correlations between levels of cytokines and flow rate •Processing methods used (length and speed of centrifuging) • Correlations between cytokine levels and demographics or health considerations

Note. BMI = body mass index. LLD = lower limit of detection.

* Some example items to assess include using brief validated screening questions: A single item ("Do you think you have dental caries [cavities] or lesions now?": (1) No, I don't think so; (2) Yes, I have pain in teeth with hot/cold foods; (3) Yes, I have a huge cavity and toothache) has a sensitivity of .85 and specificity of 1.0 for the presence of absence of dental caries upon clinical exam. A second item about deep pockets (i.e., receding gums) ("Has any dentist/ hygienist ever told you that you have deep pockets [i.e., receding gums]?") has a sensitivity of .55 and specificity of .90 (Buhlin et al., 2002). A final third question about periodontal or gum disease ("Has a dentist or dental hygienist ever told you that you had periodontal or gum disease?") has a sensitivity of .31 and specificity of .86 for moderate periodontal disease based on clinical exam (LaMonte et al., 2014).