SALIVARY IL-1β

ELISA KIT

For Research Use Only
Not for use in Diagnostic Procedures

Item No. 1-3902, (Single) 96-Well Kit;
1-3902-5, (5-Pack) 480 Wells

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SALIMETRICS®

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Intended Use

The Salimetrics® IL-1β ELISA kit is a sandwich immunoassay specifically designed and validated for the quantitative measurement of salivary IL-1β. It is not intended for diagnostic use. This assay kit was designed and optimized for salivary research use in humans. Salimetrics has not validated this kit for serum, plasma or saliva samples from any other species.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in unreliable values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

IL-1β is a key pro-inflammatory cytokine that is released after infection, injury, or antigenic challenge. It has been widely studied for its role as a multi-functional signaling molecule that affects nearly all cell types, either alone or in combination with other pro-inflammatory cytokines (1,2).

Circulating IL-1β is principally produced by activated macrophages, but it is also secreted from a wide variety of epidermal, epithelial, lymphoid, and vascular tissues (1,2). IL-1β is also present in the saliva of both healthy and diseased individuals. In the oral cavity, the sources of IL-1β production include macrophages, monocytes, fibroblasts, and mucosal epithelial cells (3,4). IL-1β has also been reported to be synthesized and released from acinar and ductal cells in mouse salivary glands (5-7). Human tear fluid and gingival crevicular fluid (GCF), which may be present as components of whole saliva, also contain IL-1β (8,9).

Salivary IL-1β has been reported to have a circadian rhythm with highest levels in the morning (after waking) and lowest levels in the evening (10-12). This is the opposite of the pattern seen in serum, where peak levels occur from 1-4 AM and daytime levels are low (13).

Levels of IL-1β in saliva and GCF have been studied in relation to gingival and periodontal disease, and significant correlation to the presence of periodontal disease has been found (8,14-19).

Levels of IL-1β in saliva and GCF have been observed to change in response to various types of physical and psychological stressors, similar to the response seen in circulation (10,20-22).

The relationship between salivary and circulating levels of IL-1β is not clear. Serum or plasma levels of IL-1β in healthy individuals are very low and often below the limit of detection (1,23,24). IL-1β levels are generally higher in saliva than in plasma or serum (23,25). One study has reported that the correlation between human passive drool saliva samples and plasma for IL-1β is not statistically significant (26).

Test Principle

This is a sandwich ELISA kit. IL-1β in standards and samples binds to the antibody binding sites on a microtitre plate. After incubation, unbound components are washed away. Biotin conjugated to goat antibodies to human IL-1β are added and attach to the bound IL-1β. After incubation, unbound components are washed away. Streptavidin conjugated to horseradish peroxidase (HRP) is added and binds to the biotin conjugated to the goat antibodies. Bound Streptavidin-HRP is measured by the reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The amount of Streptavidin-HRP detected is proportional to the amount of IL-1β present in the sample (27).

Safety Precautions

Read Safety Data Sheets before handling reagents.

Hazardous Ingredients

Liquid Stop Solution is caustic; use with care. We recommend the procedures listed below for all kit reagents.

Handling

Follow good laboratory practices when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using appropriate absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing call a physician.

The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

Safety Data Sheets are available by contacting Salimetrics at support@salimetrics.com (See www.salimetrics.com for alternative contact options).

General Kit Use Advice

- This kit uses break-apart microtitre strips. You may run less than a full plate. Unused wells must be stored at 2-8°C in the foil pouch with desiccant and used in the frame provided.
- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. ***See the Storage Section for specifications and limitations.*** Store all reagents at 2-8°C.
- The quantity of reagent provided with a single kit is sufficient for two partial runs*, if reconstituted standard and controls have been stored properly. See Storage Section for limitations.* The volumes of wash buffer, antibody conjugate and Streptavidin-HRP prepared for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
- Do not mix components from different lots of kits.
- We recommend saving all reagents until data analysis has confirmed a successful run to facilitate troubleshooting if necessary.
- Prior to sample addition, please label each strip to assure plate orientation and sample order when data is acquired on plate reader.
- To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
- When using a multichannel pipette to add reagents, always follow the same sequence when adding all reagents so that the incubation time is the same for all wells.
- When running multiple plates, or multiple sets of strips, a standard curve must be run with each individual plate and/or set of strips.
- The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68-74°F (20-23.3°C). Higher or lower temperatures may affect OD values.
- Routine calibration of pipettes and other equipment is critical for the best possible assay performance.
- When mixing plates during assay procedures, avoid speeds that spill the contents of the wells.

Storage

All unopened components of this kit are stable at 2-8°C until the kit's expiration date. ***Reconstituted standard and controls are stable for 4 hours at room temperature and for 48 hours at 2-8°C.***

Specimen Collection

Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering

sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

Collect whole saliva by unstimulated passive drool. Donors may tilt the head forward, allowing the saliva to pool on the floor of the mouth, then pass the saliva through the SalivaBio Collection Aid (SCA) into a polypropylene vial. Collection protocols/methods are available online at www.salimetrics.com or upon request.

Samples visibly contaminated with blood should be recollected. Samples may be screened for possible blood contamination (28,29) using our Blood Contamination EIA Kit (Item Nos. 1-1302/1-1302-5). Do not use dipsticks, which result in false positive values due to salivary enzymes.

Record the time and date of specimen collection.

IL-1β does not appear to be flow rate dependent in individuals with IL-1β levels in the normal range, based on the high correlation ($r(25)=0.88$, $p<0.001$, $n=27$) between measurements in pg/mL and measurements corrected for flow rate. However, the effect of flow rate in individuals with higher levels of IL-1β has not been determined. It is therefore advisable to collect data on saliva flow in case the correction for flow rate should be necessary, or to allow for future testing of archived samples for additional biomarkers that may be sensitive to flow rate. We recommend you measure the amount of time needed to collect the desired volume of saliva, in order to determine the flow rate (mL/min). The measured concentration should then be multiplied by the flow rate in order to express the result as product measured per unit of time. Protocols for flow-rate conversion are available on request.

Sample Handling and Preparation

After collection, it is important to keep samples cold in order to avoid bacterial growth and loss of IL-1β in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20ºC within 4 hours of collection. (Samples may be stored at -20ºC for up to 6 months.) However, samples held for 96 hours at 4ºC had minimal changes in salivary IL-1β levels. For long term storage, refer to the Salimetrics Collection and Handling Advice Booklet.

Do not add sodium azide to saliva samples as a preservative, as it may cause interference in the immunoassay.

On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g for 15 minutes. Freezing saliva samples will precipitate mucins. Centrifuging removes mucins and other particulate matter which may interfere with antibody binding and affect results. Samples should be at room temperature before making dilutions. Pipette clear sample into appropriate dilution tubes. Re-freeze saliva samples as soon as possible after running assay. Re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles.

Saliva samples must be diluted for this assay. See Procedure for details.

Materials Supplied with Single Kit

	Item	Quantity/Size
1	**Microtitre Plate** Coated with mouse IL-1β antibodies.	1/96 well
2	**IL-1β Standard** Lyophilized. 200 pg/mL when reconstituted to the volume stated on the Standard vial label. Prepare and serially dilute before use according to Reagent Preparation Contains: IL-1β, buffer, preservative.	1 vial
3	**IL-1β Controls** High, Low. Lyophilized. Reconstitute to 1 mL before use according to Reagent Preparation. Contains: IL-1β, buffer, preservative.	2 vials
4	**IL-1β Antibody Conjugate** Concentrate. Dilute before use with IL-1β Assay Diluent. (See Step 8 of Procedure.) Contains: Biotin conjugated to human IL-1β antibody, preservative.	1 vial / 50 µL
5	**Streptavidin-HRP** Concentrate. Dilute before use with IL-1β Assay Diluent. (See step 11 of procedure.) Contains: Streptavidin conjugated to HRP, preservative.	1 vial / 100 µL
6	**IL-1β Sample Diluent** Contains: phosphate buffer, preservative.	1 bottle / 30 mL
7	**IL-1β Assay Diluent** Contains: Buffer with protein stabilizer, preservative.	1 bottle / 30 mL
8	**Wash Buffer Concentrate (10X)** Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle / 100 mL
9	**TMB Substrate Solution** Non-toxic, ready to use.	1 bottle / 25 mL
10	**Stop Solution**	1 bottle / 12.5 mL
11	**Adhesive Plate Covers**	2

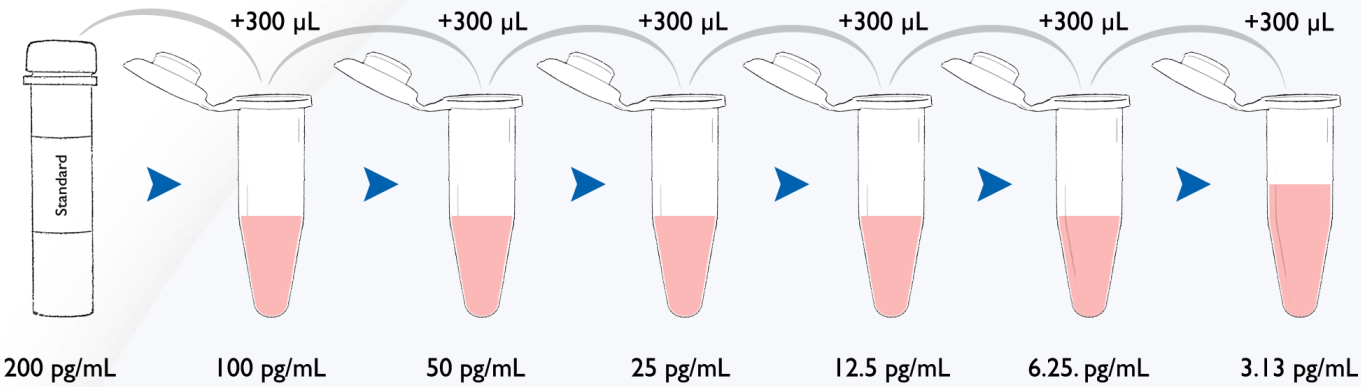
Materials Needed But Not Supplied

- Precision pipette to deliver 20 µL to 300 µL
- Precision multichannel pipette to deliver 50 µL and 100 µL
- Vortex
- Plate rotator with 0.08-0.17 inch orbit capable of 500 rpm
- Plate reader with 450 nm and 620 to 630 reference filters
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- Two disposable polypropylene tubes to hold at least 12 mL
- Small disposable polypropylene tubes for dilution of standard and samples
- Pipette tips
- Serological pipette to deliver up to 12 mL
- Centrifuge capable of 1500 x g

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is recommended for the 12 mL of IL-1β Assay Diluent used in Step 8 and 11 to come to room temperature.
- Bring microtitre plate to room temperature before use. ***It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.***
- Prepare 1X wash buffer by diluting Wash Buffer Concentrate (10X) 10-fold with room-temperature deionized water (100 mL of Wash Buffer Concentrate (10X) to 900 mL of deionized water). ***Dilute only enough for current day's use and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay.)
- Reconstitute each Control vial with 1.0 mL of deionized water. (We recommend sterile water if you plan to store at 2-8°C.) Let sit 20 minutes at room temperature before using. Mix well immediately before use. ***Use reconstituted controls within 4 hours at room temperature or refrigerate at 2-8°C for up to 48 hours.***

- Reconstitute IL-1β Standard with deionized water according to the volume on the standard vial label. (We recommend sterile water if you plan to store at 2-8°C.) Let sit 20 minutes at room temperature before using. Mix well immediately before use. ***Use reconstituted standard within 4 hours at room temperature or refrigerate at 2-8°C for up to 48 hours.***
- Prepare serial dilutions of the IL-1β Standard as follows:
    - Label six polypropylene microcentrifuge tubes or other small tubes 2 through 7.
    - Pipette 300 µL of IL-1β Assay Diluent into tubes 2 through 7. ***Do not use IL-1β Sample Diluent to dilute the standard curve.***
    - Serially dilute the standard 2X by adding 300 µL of the 200 pg/mL standard (tube 1) to tube 2. Mix well.
    - After changing pipette tips, remove 300 µL from tube 2 to tube 3. Mix well.
    - Continue for tubes 4, 5, 6 and 7.
    - The final concentrations of standards for tubes 1 through 7 are, respectively, 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL and 3.13 pg/mL. Standard concentrations in pmol/L are 11.8, 6.0, 3.0, 1.5, 0.7, 0.4 and 0.2 respectively.
    - IL-1β Assay Diluent is used for the Zero Standard.



Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. (Standards, controls, and saliva samples should be assayed in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	200 Std	200 Std	Ctrl-H	Ctrl -H								
B	100 Std	100 Std	Ctrl-L	Ctrl -L								
C	50 Std	50 Std	SMP-1	SMP-1								
D	25 Std	25 Std	SMP-2	SMP-2								
E	12.5 Std	12.5 Std	SMP-3	SMP-3								
F	6.25 Std	6.25 Std	SMP-4	SMP-4								
G	3.13 Std	3.13 Std	SMP-5	SMP-5								
H	0 Std	0 Std	SMP-6	SMP-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Step 3: Pipette 12 mL of IL-1β Assay Diluent into each of two different disposable tubes. (Scale down proportionally if not using the entire plate.) Set aside for Step 8 and Step 11.

Step 4: Dilute saliva samples 15X in IL-1β Sample Diluent using 20 µL saliva to 280 µL IL-1β Sample Diluent. ***Do not dilute samples in IL-1β Assay Diluent.***

Step 5:

- Pipette 100 µL of standards, controls, and diluted saliva samples into appropriate wells.
- Pipette 100 µL of IL-1β Assay Diluent into 2 wells to serve as the Zero Standard.

Step 6: Place adhesive cover provided over plate. Mix plate on a plate rotator ***continuously*** at 500 rpm for 1 hour at room temperature.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 8: Dilute the antibody conjugate 1:500 by adding 24 µL of the antibody conjugate to the 12 mL of IL-1β Assay Diluent. (Scale down proportionally if not using the entire plate.) Antibody conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted antibody conjugate solution and add 100 µL to each well using a multichannel pipette.

Step 9: Place a new adhesive cover (provided) over plate. Mix plate on a plate rotator ***continuously*** at 500 rpm for 2 hours at room temperature.

Step 10: Repeat wash procedure from Step 7.

Step 11: Dilute the Streptavidin-HRP 1:200 by adding 60 µL of the Streptavidin-HRP to the 12 mL of IL-1β Assay Diluent. (Scale down proportionally if not using the entire plate.) Streptavidin-HRP tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted Streptavidin-HRP solution and add 100 µL to each well using a multichannel pipette.

Step 12: Mix plate on a plate rotator ***continuously*** at 500 rpm for 20 minutes at room temperature.

Step 13: Repeat wash procedure from Step 7.

Step 14: Add 100 µL of TMB Substrate Solution to each well with a multichannel pipette.

Step 15: Mix plate on a plate rotator ***continuously*** at 500 rpm in the dark (covered) for 20 minutes at room temperature.

Step 16: Add 50 µL of Stop Solution with a multichannel pipette.

Step 17:

- Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.

  Caution: Spillage may occur if mixing speed exceeds 600 rpm.

- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. (For best results, a secondary filter correction at 620 to 630 nm is recommended.)

Quality Control

The Salimetrics' High and Low IL-1β Controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Determine the concentrations of the controls and saliva samples by interpolation using data reduction software. We recommend using a 4-parameter non-linear curve fit.
3. Multiply the calculated concentrations of the saliva samples by the dilution factor of 15 to obtain final IL-1β concentrations in pg/mL.
4. Samples (diluted 15X) with IL-1β values greater than 200 pg/mL (or >3000 pg/mL after multiplying by the dilution factor of 15) should be diluted further with IL-1β Sample Diluent and rerun for accurate results. If a dilution of the sample is used, multiply the results by the additional dilution factor.

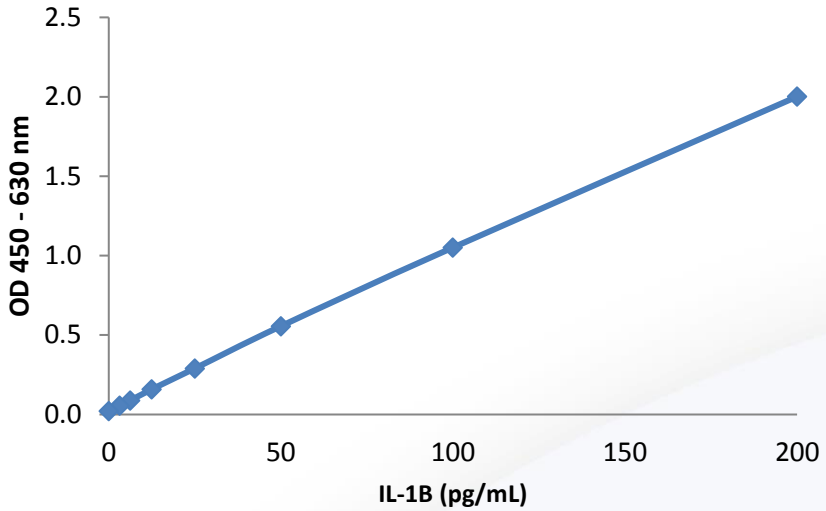
A new Standard Curve must be run with each full or partial plate.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Well	Standard	Average OD	IL-1β (pg/mL)
A1,A2	S1	2.001	200
B1,B2	S2	1.051	100
C1,C2	S3	0.556	50
D1,D2	S4	0.290	25
E1,E2	S5	0.159	12.5
F1,F2	S6	0.088	6.25
G1,G2	S7	0.056	3.13
H1,H2	Zero	0.021	0

Example: IL-1β 4-Parameter Curve Fit



Limitations

- Samples (diluted 15X) with IL-1β values greater than 200 pg/mL (or >3000 pg/mL after multiplying by the dilution factor of 15) should be diluted further with IL-1β Sample Diluent and rerun for accurate results. If a dilution of the sample is used, multiply the results by the additional dilution factor.
- See "Specimen Collection" recommendations to ensure proper collection of saliva specimens and to avoid interfering substances.
- Samples collected with sodium azide are unsuitable for this assay.
- Any quantitative results indicating abnormal IL-1β levels should be followed by additional testing and evaluation.

Salivary IL-1β Example Ranges*

Group	N	Mean (pg/mL)	Mean (x15 Dilution) (pg/mL)	Standard Deviation (pg/mL)	Mean adjusted for flow rate (pg/min.)	Correlation of Mean (x15 Dilution) to Flow Rate
Adults	27	8	122	138	72	-0.184

*To be used as a guide only. Each laboratory should establish its own range.

Salivary IL-1β ELISA Kit Performance Characteristics

Precision

The intra-assay precision was determined from the mean of 20 replicates each.

Saliva Sample	N	Mean (pg/mL)	Mean (x15 Dilution) (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
1	20	116	1740	31	2
2	20	65.2	977	19	2
3	20	5.87	88.0	2	3
4	20	23.0	345	8	2
5	20	9.66	145	4	3

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

Saliva Sample	N	Mean (pg/mL)	Mean (x15 Dilution) (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
1	20	17.1	256	11	4
2	20	56.9	853	52	6
3	20	27.7	415	11	3
4	20	106	1595	71	4
5	20	6.75	101	3	3
6	20	107	1599	106	7

Recovery

Four saliva samples containing different levels of an endogenous IL-1β were spiked with known quantities of IL-1β and assayed.

Saliva Sample	Endogenous (pg/mL)	Endogenous (x15 Dilution) (pg/mL)	Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Observed (x15 Dilution) (pg/mL)	Recovery (%)
1	0.53	8	2250	2258	120.02	1800	80
	0.53	8	1250	1258	72.75	1091	85
	0.53	8	375	383	23.93	359	94
2	1.25	19	2250	2269	125.53	1883	83
	1.25	19	1250	1269	81.01	1065	82
	1.25	19	375	394	22.51	338	85
3	34.99	540	2250	2790	151.38	2271	80
	34.99	540	1250	1790	106.58	1599	86
	34.99	540	375	915	51.21	768	69
4	20.35	305	2250	2555	151.94	2279	89
	20.35	305	1250	1555	98.44	1477	93
	20.35	305	375	680	43.17	648	94

Sensitivity

Analytical Sensitivity

The lower limit of sensitivity was determined by interpolating the mean optical density plus 2 SDs of 10 sets of duplicates at the 0 pg/mL level. The minimal concentration of IL-1β that can be distinguished from 0 is <0.37 pg/mL (6 when multiplied by the x15 dilution factor).

Functional Sensitivity

The functional sensitivity was determined by assaying 20 samples at a concentration level resulting in a CV of 20%. The functional sensitivity of the salivary IL-1β ELISA is 0.6 pg/mL (9 pg/mL when multiplied by the x15 dilution factor).

Drift

Drift was determined by individually pipetting 96 wells of one IL-1β concentration across the plate and determining the CV of the optical densities for all wells. The CV resulting from this calculation was 3.7%.

Linearity of Assay

Two saliva samples were serially diluted with each other proportionately and assayed.

Saliva Sample	Dilution Factor	Expected (pg/mL)	Observed (pg/mL)	Observed (x15 Dilution) (pg/mL)	Recovery (%)
1			1.80	26.94	
	1:9	12.92	11.19	167.78	87
	2:8	24.05	26.15	392.30	109
	3:7	35.18	36.28	544.26	103
	4:6	46.30	46.25	693.74	100
	5:5	57.43	54.78	821.71	95
	6:4	68.56	67.55	1013.26	99
	7:3	79.68	74.24	1113.65	93
	8:2	90.81	86.60	1299.03	95
	9:1	101.94	98.88	1483.17	97
2			113.06	1695.96	

Antibody Specificity

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in Salivary IL-1β-ELISA
Human IL-1α	50	0
Rat IL-1α	50	0
Rat IL-1β	6.25	3.8
Mouse IL-1α	50	0
Mouse IL-1β	50	0
Porcine IL-1α	50	0
Porcine IL-1β	50	0

References

1. Dinarello, C.A. (1996). Biologic basis for interleukin-1 in disease. *Blood, 87*(6), 2095-147.
2. Dinarello, C.A. (2002). The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol, 20*(27), S1-S13.
3. Giannobile, W.V., Beikler, T., Kinney, J.S., Ramseier, C.A., Morelli, T., & Wong, D.T. (2009). Saliva as a diagnostic tool for periodontal disease: Current state and future directions. *Periodontology 2000, 50,* 52-64.
4. Perrier, S., Kherratia, B., Deschaumes, C., Ughetto, S., Kemeny, J.L., Baudet-Pommel, M. & Sauvezie, B. (2002). IL-1ra and IL-1 production in human oral mucosal epithelial cells in culture: Differential modulation by TGF-β1 and IL-4. *Clin Exp Immunol, 127*(1), 53-9.
5. Seil, M., El Ouaaliti, M., Abdou Foumekoye, S., Pochet, S., & Dehaye, J.P. (2012). Distinct regulation by lipopolysaccharides of the expression of interleukin-1 by murine macrophages and salivary glands. *Innate Immun, 18*(1), 14-24.
6. Yao, C., Karabasil, M.R., Purwanti, N., Li, X., Akamatsu, T., Kanamori, N., & Hosoi, K. (2006). Tissue Kallikrein mK13 is a candidate processing enzyme for the precursor of interleukin-1β in the submandibular gland of mice. *J Biol Chem, 281*(12), 7968-76.
7. Tanda, N., Ohyama, H., Yamakawa, M., Ericsson, M., Tsuji, T., McBride, J., Elovic, A., Wong, D.T., Login, G.R. (1998). IL-1 beta and IL-6 in mouse parotid acinar cells: Characterization of synthesis, storage, and release. *Am J Physiol, 274*(1 Pt 1), G147-56.
8. Miller, C.S., Foley, J.D., Bailey, A.L., Campell, C.L., Humphries, R.L., Christodoulides, N., Floriano, P.N., et al. (2010). Current developments in salivary diagnostics. *Biomark Med, 4*(1), 171-89.
9. Solomon, A., Dursun, D., Liu, Z., Xie, Y., Macri, A., & Pflugfelder, S.C. (2001). Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. *Invest Ophthalmol Vis Sci, 42*(10) 2283-92.
10. Ilardo, C., Toniolo, A.M., Aimone-Gastin, I., Abdelmouttaleb, I., Guéant, J.L., & Desor, D. (2001). Effects of psycho-physical stress (competitive rafting) on saliva interleukin-1 beta. *Stress Health, 17*(1), 9-15.
11. Chiappelli, F., Iribarren, F.J., & Prolo, P. (2006). Salivary biomarkers in psychobiological medicine. *Bioinformation, 1*(8), 331-34.
12. Reinhardt, E.L., Fernandes, P.A., Markus, R.P., & Fischer, F.M. (2012). Daily rhythm of salivary IL-1β, cortisol and melatonin in day and night workers. *Work, 41*(Suppl. 1), 5788-90.
13. Petrovsky, N. (2001). Towards a unified model of neuroendocrine-immune interaction. *Immunol Cell Biol, 79*(4), 350-57.
14. Han, J.Y. & Reynolds, M.A. (2012). Effect of anti-rheumatic agents on periodontal parameters and biomarkers of inflammation: A systematic review and meta-amalysis. *J Periodontal Implant Sci, 42*(1), 3-12.
15. Lee, A., Ghaname, C.B., Braun, T.M., Sugai, J.V., Teles, R.P., Loesche, W.J., Kornman, K.S., Giannobile, W.V., & Kinney, J.S. (2012). Bacterial and salivary biomarkers predict the gingival inflammatory profile. *J Periodontol, 83*(1), 79-89.
16. Fitzsimmons, T.R., Sanders, A.E., Bartold, P.M., & Slade, G.D. (2010). Local and systemic biomarkers in gingival crevicular fluid increase odds of periodontitis. *J Clin Periodontol, 37*(1), 30-36.
17. Tobón-Arroyave, S.I., Jaramillo-González, P.E., & Isaza-Guzmán, D.M. (2008). Correlation between salivary IL-1β levels and periodontal clinical status. *Arch Oral Biol, 53*(4), 346-52.

18. Miller, C.S., King, C.P., Jr., Langub, M.C., Kryscio, R.J., & Thomas, M.V. (2006). Salivary biomarkers of existing periodontal disease: A cross-sectional study. *J Am Dent Assoc, 137*(3), 322-29.

19. Christodoulides, N., Floriano, P.N., Miller, C.S., Ebersole, J.L., Mohanty, S., Dharshan, P., Griffin, M., et al. (2007). Lab-on-a-chip methods for point-of-care measurements of salivary biomarkers of periodontitis. *Ann N Y Acad Sci, 1098*, 411-28.

20. Zefferino, R., Facciorusso, A., Lasalvia, M., Narciso, M., Nuzzaco, A., Lucchini, R., & L'Abbate, N. (2006). Salivary markers of work stress in an emergency team of urban police (1° step). *G Ital Med Lav Ergon, 28*(4), 472-77.

21. Deinzer, R., Granrath, N., Stuhl, H., Twork, L., Idel, H., Waschul, B., & Herforth, A. (2004). Acute stress effects on local IL-1 responses to pathogens in a human in vivo model. *Brain Behav Immun, 18*(5), 458-67.

22. Dugué, B., Ilardo, C., Aimone-Gastin, I., Guéant, J.L., Mouzé-Amady, M., Cnockaert, J.C., Mur, J.M., et al. (1996). Cytokines in saliva. Basal concentrations and the effect of high ambient heat (sauna). *Stress Med, 12*(3), 193-97.

23. Brailo, V., Vucicevic-Boras, V., Lukac, J., Biocina-Lukenda, D., Zilic-Alajbeg, I., Milenovic, A., & Balija, M. (2012). Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. *Med Oral Patol Oral Cir Bucal, 17*(1), e10-5.

24. Wong, H.L., Pfeiffer, R.M., Fears. T.R., Vermeulen, R., Ji, S., & Rabkin, C.S. (2008). Reproducibility and correlations of multiplex cytokine levels in asymptomatic persons. *Canc Epidemiol Biomarkers Prev, 17*(12), 3450-56.

25. Llamas Moya, S., Boyle, L.A., Lynch, P.B., & Arkins, S. (2006). Pro-inflammatory cytokine and acute phase protein responses to low-dose lipopolysaccharide (LPS) challenge in pigs. *Animal Sci, 82*, 527-34.

26. Williamson, S., Munro, C., Pickler, R., Grap, M.J., & Elswich, R.K., Jr. (2012). Comparison of biomarkers in blood and saliva in healthy adults. *Nurs Res Pract*, 246178.

27. Chard, T. (1990). *An introduction to radioimmunoassay and related techniques* (4th ed.). Amsterdam: Elsevier.

28. Kivlighan, K. T., Granger, D.A., Schwartz, E.B., Nelson, V., Curran, M., & Shirtcliff, E.A. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Horm Behav, 46*(1), 39-46.

29. Schwartz, E., & Granger, D.A. (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clin Chem, 50*(3), 654-56.

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