Integrating Salivary Analytes into Behavioral, Developmental and Health Science Research

2015
History

- Salimetrics was founded in 1998 in a laboratory at Penn State University, by researchers for researchers.

- Now located in State College, PA and Carlsbad, CA with Salimetrics Europe in the UK, and global partners.
Around the world

- Salimetrics Hq
- Salimetrics Europe
- Salimetrics Partner Lab
- Salimetrics Distributors
- Center for Interdisciplinary Salivary Bioscience Research
We are science. We enable research.
Integrating Saliva

Step 1: Researching Saliva Biomarkers
Step 2: Preparing to Collect Saliva
Step 3: Collecting Saliva (Field or Lab)
Step 4: Organizing, Aliquoting and Storing Samples
Step 5: Transporting Samples for Testing
Step 6: Selecting Assay Kits
Step 7: Testing Samples for Biomarkers and DNA
Step 8: Reviewing Accuracy of Testing Results
Step 9: Preparing Samples for Long-Term Storage
Step 10: Analyzing Saliva Biomarker Data
Publish Findings

Key:
= Salimetrics Products and Services

www.salimetrics.com
Agenda

• Assumptions and overviews
• Oral Fluids as Biological Specimens
• Collection Demonstration
• Research Design & Sampling Schemes
• How the Assay works
• Future Directions
Effects of context on development moderated through individual differences in stress responsive biological systems.

Biology and behavior have reciprocal effects and expression of bio-behavioral relationships is dependent on context.

Probability that individual differences in biological reactivity and regulation linked to outcomes of interest highest when studied in meaningful social contexts.

Biological systems are networked and multi-system measurement of stress response is critical.
Basic concept: context

Environmental Demands
(physical, social, cultural)

Behavioral Surface
(emotion regulation, coping, flight-fight, tend-befriend)

Fast Acting—
Physiological Processes
(neural, HPA, ANS activity)

Slow Acting—
Physiological Processes
(genetic activity)

Micro-evolution and individual development (Gottlieb, G.)

Intra-individual patterns of stress-reactivity

(McEwen, B.)

• Normative-adaptive pattern assumes habituation of reactivity and recovery over time

• Four Basic Maladaptive patterns:
  – Chronic hyper-reactivity
  – Failure to habituate
  – Dysregulated recovery
  – Hypo-reactivity
Intra-individual patterns of stress-reactivity

Normative-adaptive pattern assumes habituation of reactivity and recovery over time

McEwen, 1998
Maladaptive Patterns of Stress Reactivity

Dysregulated Recovery

Failure To Habituate

Time

Exposure History

McEwen
Maladaptive Patterns of Stress Reactivity

Chronic Hyperactivity

Chronic Hypo-reactivity

Context Type

A  B  C  D  E
Biological Sensitivity to Context and Health Disparities

Social Contextual Demands and Environmental Forces

Behavior genetics
Physiology

Intrinsic Individual Differences in Development

Infancy
Childhood
Adulthood
Biology of oral fluids
## Advantages of oral fluid for research

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Minimally Invasive”</td>
<td>Considered “acceptable and non-invasive” by research participants. Collection is quick, non-painful, uncomplicated</td>
</tr>
<tr>
<td>“Safety”</td>
<td>Reduces transmission of infectious disease by eliminating the potential for accidental needle sticks. CDC does not consider saliva a class II Biohazard unless visibly contaminated with blood (Check with your institution)</td>
</tr>
<tr>
<td>“Self-collection”</td>
<td>Allows for community- and home-based collection. Enables specimen collection in special populations</td>
</tr>
<tr>
<td>“Economics”</td>
<td>Eliminates the need for a health care intermediary (e.g., phlebotomist, nurse). Resources for collection and processing samples are low cost and available</td>
</tr>
<tr>
<td>“Accuracy”</td>
<td>Salivary levels of many analytes represent the “free unbound fraction” or biological active fraction in the general circulation</td>
</tr>
<tr>
<td>“Ecological Validity”</td>
<td>Enables biological reactivity and regulation to be monitored in everyday social world</td>
</tr>
</tbody>
</table>

---

Slavkin
Basic functions of oral fluids

- Lubricate oral cavity
- Early degradation of food
- Protect teeth against acid, bacteria, viruses, and fungi
- Maintain mineral content of tooth enamel
Composition of Oral Fluid

• Principally water and electrolyte mixture
• pH range 4 – 8
• Salivary proteome (>1,000 analytes)
• Bronchial and nasal secretions
• Blood and serum from injury
• Bacteria, viruses, fungi
• Cellular components and food debris
Processes related to Secretion of Oral Fluid

- Day-night cycle
- ANS activation
- Taste
- Smell
- Chewing
Sources of oral fluids

- Parotid saliva (24%)
- Submandibular saliva (70%)
- Sublingual saliva (4%)
- Crevicular fluid (1-2%)
Effect of sampling location

sAA Varies by oral fluid subtype (not cortisol)

Note: sAA is flow rate dependent
Pathway of saliva biomarkers

Acinar cells

neutral molecules: passive diffusion – cortisol, DHEA, sex steroids, melatonin, etc. (1)

DHEA-S: ultrafiltration (or active transport ?) (2)

SIgA: active transport (3)

proteins: synthesis – sAA (3)

(1) Not affected by location or flow
(2) Affected by flow
(3) Affected by location and flow
Movement of substances from blood into oral fluid

- **Passive diffusion** via capillaries surrounding salivary glands
- **Filtering** thru tight spaces between cells in the salivary glands
- **Outflow** of serum-like crevicular fluid between teeth and gums
- **Active transport** through the secretory cells of the glands
Numerous capillaries surround the saliva glands. Hormones and other compounds pass through the capillary walls and bathe the salivary glands.

Cortisol is secreted into the bloodstream from the adrenal cortex. About 95% of it binds to carrier proteins (binding globulin). Only the remaining 5% is available for use by target tissues.

Unbound cortisol is lipid soluble and can move through the lipo-protein cell membranes of the secretory cells by passive diffusion. It is then released into the saliva.
Pathway of saliva biomarkers

Proposed Entry Pathways for CRP into Saliva

- CRP in GCF flows into saliva
- Local CRP production from gingival tissues?
- Local Infections (e.g., Periodontitis)
- Systemic Infections
- Tissue Damage, etc.
- Immune cells and tissues release inflammatory mediators: IL-6, IL-1β, TNF-α
- Circulating CRP leaks into GCF through gingival tissues
- Liver cells increase production of CRP
Normative Sources of Variability in Salivary Analyte Levels

Flow Rate Dependence
Large blood borne molecules (ie. DHEA-S)
Analytes released by salivary glands (i.e., sIgA, sAA)

Techniques to record/control [concentration/volume/min]
(a) set standard collection time, and record volume
(b) set standard volume of donation, and record time

Level = Concentration/volume (pg/mL)
Flow rate = Volume/minute (mL/min)
Output = pg/mL x mL/min = Concentration/min (ug/min)
Pre-Sample Collection Issues

Oral stimulants - change sample pH, interfer binding in assays

Food and drink - leave residue, particulate matter, change specimen composition, interfere with liquid handling

Diary products - bovine hormones, cross-react with antibodies used in assays

**Solution**: Rinse mouth with water

**Caveat**: Rinsing will dilute concentration/volume units thus delay sample collection for 10 minutes
Blood Contamination in Saliva

- Leakage of blood and its products into oral cavity has potential to compromise measurements of salivary analytes
- Poor oral health and periodontitis
  - Age, access to health care, iatrogenic effect, systemic infection or inflammation, substance and tobacco use
- Dental work, oral injury, shedding or loosing teeth
- Granger et al (2007), Prevalence, characteristic of samples, effect size relative to statistical power

Solution:
- Screening
- Regular hygiene?
- Access to Oral Care
- Gums bleed when brush or floss?
- Rating scale for discoloration
- Exclude samples visibly contaminated
- Salivary ‘Transferrin’
Scaling Blood in Saliva

- ‘1’ - Appears clear, no visible color
- ‘2’ - A hint of color, a little brown or yellow tint is barely visible
- ‘3’ - Clearly visible yellow or brown tint
- ‘4’ - Yellow or brown coloring is more than just a tint, color is obvious but not very deep
- ‘5’ - Very colored, deep, rich, dark yellow or brown is very apparent


Medications and Salivary Cortisol

- Effects subjective experience of stress, novelty, threat or pain
- Agonist or antagonist of specific HPA secretagogues
- Effects Synthesis or Release of Cortisol from Adrenal
- Effects a Physiological System Networked with HPA
- Effects Composition of Saliva
- Effects Availability of Saliva
- Effects Transport of Serum Constituents into Oral Fluid
- Effects CBG Levels or Binding to Cortisol
- Specifically Cross-reacts with anti-cortisol antibody
- Causes Non-specific Interference in relation to cortisol-antibody binding
- Intra-nasal or oral administration as either an inhalant or topical gel

Substance Use

• Nicotine - HPA and ANS stimulant

• Tobacco smoke - poor oral health, acid aldehydes

• Alcohol – respiration into oral fluid

• Illegal substances - “no comment”

• Medications – 4,000+
Some Exclusion Criteria

- Anti-inflammatory steroid use
- Cytokine-based treatments (interferon)
- Radiation of salivary glands
- Cushings or Addison’s diseases
- Systemic infection (fever > 101 F)
- Upper respiratory infection (nasal drainage)
- Inflammation - sore throat, periodontitis
Infection and the HPA System

Boonstra, 1998


Fever
Nasal Secretions
Sore Throat
What can you study with saliva?
Analytes in Oral Fluid of Interest to Developmental and Health Science

- Cortisol
- Dehydroepiandrosterone
- Testosterone
- Estradiol, estrone, estriol
- sIgA
- Progesterone and 17-alpha-hydroxy-Progesterone
- Cotinine
- Drugs of Abuse and Environmental Chemicals
- Uric Acid
- C-Reactive Protein, Neopterin, Beta-2-microglobulin
- Melatonin, Oxytocin, Andiponectin
- Cytokines (e.g., IL-6, TNFa, IL1b), soluble cytokine receptors (sTNF-I, -II)
- Disease specific antibodies (e.g., HIV, HSV, EBV, CMV) or antigens
- Alpha-amylase, Chromogranin A
- Metalloproteinases (MMP-8)
- Heat Shock Proteins, aldosterone
- Electrolytes
- DNA and Methylation, RNA
Publication Trends in Salivary Bioscience

Salivary proteins and peptides

Studies with children and adolescents
Sample collection, handling and storage
Collection techniques

Passive drool
Absorbent swabs
Microsponge spears
Filter paper

Passive drool
Absorbent swabs
Microsponge spears
Filter paper

Oral fluid (whole saliva)

- Sublingual saliva
- Parotid saliva
- Submandibular saliva
- Crevicular fluid
Collection exercise!
Collection exercise!
Passive drool to collect whole saliva

- Imagine chew food
- Move jaws gently
- Allow saliva to pool under tongue
- Resist swallowing reflex
- Gently force saliva thru SCA into collection vial
Passive Drool: advantages and disadvantages

- Large volume in short time
- Low cost
- Sample volume confirmation in field
- Unlimited analysis potential
- Requires skill and compliant participant
- Unfiltered specimen

www.salimetrics.com
Salimetrics Oral & Children’s Swabs

- Easy to use
- Excellent Recovery
- 1.5 mL capacity

Approved for AA, Cortisol, Cotinine, CRP, Chromagranin A, Testosterone, sIgA, IL1-β, IL-6, Melatonin and Uric Acid

Ability to Target the Location of placement

Swab material is controlled by QC analysis; can use for longitudinal studies

www.salimetrics.com
Tips for younger children

- Infants – collect for a total of 60-90 seconds
- Position the swab between check and gum line where the saliva pools for infants
- Sing familiar song, count, ABCs
- Egg Timer
- Model
- Dim light conditions - Pen light
- 1 Year – Stranger awareness, parent collection
Selecting a collection technique

- Participant age
- Volume of sample available and needed (QNS)
- Single or multi-analyte measurement
- Participant burden
- Number of samples needed
- Self- or assisted collection
- Test and discard
- Archive samples for future
Sample storage

- Freeze thaw cycle
- Field storage
- Long term storage and archiving
  - Aliquoting & labeling vs. temperature
  - Organization and communication with different coordinators

www.salimetrics.com
DNA collection & handling
DNA collection & handling

VOLUME

COLLECTION TECHNIQUE

STORAGE TEMPERATURE

MULTIPLE FREE-THAW CYCLES

COLLECTION LOCATION

*BMC Medical Research Methodology.*

www.salimetrics.com
1. A small initial sample volume (100µl) may be adequate to recover DNA sufficient for multiple genetic analyses (using standard 5-10ng per assay).

2. Quality and quantity data indicate whole saliva returns the greatest quantity of DNA, but some collection devices tested can provide DNA of similar quality. Can be obtained from most collection techniques.

3. DNA concentration is not significantly affected by room temperature storage in the short run. DNA quality measures increase modestly with length of RT storage. Can be shipped through the mail.

4. DNA concentration is not significantly affected by number of Freeze/Thaw cycles. Can be taken from same sample tested for saliva biomarkers without additional handling.

5. Oral sampling location does not have an effect on DNA concentration. With consent, can be taken from prior, saved samples.
DNA expected values

Table 3. Means (Standard Deviations) for DNA Concentration and Quality Obtained From Different Sample Volumes of Whole Saliva

<table>
<thead>
<tr>
<th>Measure</th>
<th>Sample Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.10 ml</td>
</tr>
<tr>
<td>DNA concentration (ng/μl)</td>
<td>28.25 (15.36)</td>
</tr>
<tr>
<td>DNA quality (260/280 nm)</td>
<td>1.78 (.10)</td>
</tr>
<tr>
<td>DNA total amount (μg)</td>
<td>1.43 (.77)</td>
</tr>
</tbody>
</table>

10 – 20 ng/μL working stock dilutions
Each SNP assay uses 5 – 10 ng DNA
Saliva sampling schemes (examples)
Basic concept: context

Environmental Demands
(physical, social, cultural)

Behavioral Surface
(emotion regulation, coping, flight-fight, tend-befriend)

Fast Acting—
Physiological Processes
(neural, HPA, ANS activity)

Slow Acting—
Physiological Processes
(genetic activity)

Individual Development


Saliva Biomarkers

www.salimetrics.com
Study Designs

- Basal levels
- Trait vs. state
- Individual differences in reactivity and recovery to acute events
- Person-oriented approach for identifying reactivity and recovery
- Patterns of reactivity and recovery across multiple occasions
- Dissociation between behavior and HPA activity
- Diurnal rhythm and awakening response
Sampling schemes

• Awakening response
  – Waking and 30 min post
  – Monitoring compliance
  Text Messaging
  MEMS caps
  – Number of days
  – Profiling


Baseline awakening cortisol levels at 35/36 weeks gestation.

Sampling schemes

- Diurnal rhythm
  - Non-linear
  - Number of samples
  - Number of days
Mean levels of salivary cortisol (ug/dL) Rise and then fall across the day.
sAA (U/mL) diurnal pattern is opposite from cortisol: Mean levels fall and then rise across the day.
Cortisol Awakening Response: Diurnal (Example)
Between-subject differences in salivary CAR (ug/dL)
Diurnal Cort sAA (Adult pregnancy)

Figure 1a. Diurnal sAA and Cortisol as a Function of GA

Mean levels cycle across the day

Secretory IgA (example)

**Immune system and low-quality child care**

- **Log SIgA (µg/mL)**
- **Time (hours)**: 6, 11, 15, 18

- **Red**: High Sensitive Care
- **Green**: Low Sensitive Care

Developmental differences (Infant-toddler)

sAA response to Inoculation Stress

- 2-month old infants did not show an sAA response
- 6 and 12-month old infants displayed a significant sAA increase.
- 24-month old infants displayed an anticipatory rise and decrease in sAA


www.salimetrics.com
Maternal Engagement in Early Infancy Predicts Children’s Cortisol Reactivity at 15 months.

Stress Reactivity (Sampling example)

Onset STRESS
- Videos
  - 20-25"
- Speech/ IP 1
  - 10"
- Math/ IP 2
  - 5"
- Saliva 1
  - 15-20 min prior to Stress Onset
- Saliva 2
  - +10 min after Stress Onset

Offset STRESS
- Tracing/ IP 3
  - 2 min
- Questionnaires/ Videos
  - 60"
- Saliva 3
  - +17-21 min after Stress Onset
- Saliva 4
  - +27-30 min after Stress Onset
- Saliva 5
  - +35-45 min after Stress Onset
- Saliva 6
  - +73-82 min after Stress Onset

www.salimetrics.com
Dampened sAA and Cortisol Reactivity to Psychosocial Stress: Maltreated versus Comparison Adolescents

Individual Difference: posturing response

Mean changes in dominance hormone testosterone following high-power and low-power poses. (Difference scores)

Mean changes in the stress hormone cortisol following high-power and low-power poses. (Difference scores)

Cortisol, Alpha-amylase, and subjective emotional reactivity in women with Borderline personality disorder
(Scott, Granger, Levy, 2013)

The BPD group had higher average baseline and overall average NA than both NTM and TM groups.

The BPD group showed attenuated stress-related cortisol reactivity as compared to both NTM and TM groups.

Both the BPD and TM groups demonstrated attenuated stress-related sAA reactivity as compared to the NTM group.
Dyads: Caregivers of PC patient

Salivary CRP

Salivary Cortisol Response to Rowing Ergometer Competition

Men and women’s endocrine responses were more different than alike and varied by level of experience.

Individual differences associated with social Affiliation rather than dominance or competitiveness.

Kivlighan, Granger, Booth (2005). Psychoneuroendocrinology
Salivary sAA Response to Rowing Ergometer Competition

sAA higher for varsity than novice, and associated with performance. sAA reactivity associated with perceived dominance.

Kivlighan & Granger (2006) Psychoneuroendocrinology
Mucosal IgA and URTI in American College Football Players: A Year Longitudinal Study

Mucosal IgA and URTI in American College Football Players: A Year Longitudinal Study

A longitudinal study of changes in s-IgA and respiratory illness in athletes

• 38 members of America’s Cup yacht crew
• Studied over 50 weeks of training and sailing
• Morning saliva samples collected weekly
• Clinically confirmed illness

Relative s-IgA before and after an infection episode

Gleeson, Sports Medicine Conclusions

• On a group basis, relative s-IgA determined a substantial proportion of the variability in weekly infection incidence

• Significant reduction in s-IgA in the 3 weeks prior to infection

• Relative s-IgA value <40% of healthy baseline value indicated a 50% chance of contracting an infection within 3 weeks

Possible predictive value with regular monitoring

Monitoring Performance hormones

• Cortisol (C) and Testosterone (T)
• Saliva values lower but reflect free plasma levels
• With stress: \( \uparrow \) C and \( \downarrow \) T
• C/T ratio \( \uparrow \) 30% = Overreached/Stressed
• Salivary C/T \( (1000*\text{nM/pM}) \) ratio > 40
  = Stressed or Not Recovered
• May help in recovery prescription for individual players
• S-C/T >40 likely associated with underperformance

Possible value with monitoring during intensive phases of season

With permission, Professor Mike Gleeson
Mean salivary cortisol responses (±S.D.) to the Mannheim Multicomponent Stress Test (MMST).
-20–0min—baseline, 0–5min—stress induction.
The stars indicate significant differences between two measurements as assumed by post-hoc comparison tests with Bonferroni correction. *p≤0.05, **p≤0.01.

Measuring Saliva Analytes
Test Principle

- Microtiter plate coated with monoclonal antibodies
- Cortisol in standards, controls, and unknowns compete with cortisol linked to HRP for antibody binding sites
- Unbound components are washed away
- Bound Cortisol-HRP reacts with TMB generating a blue color
- Sulfuric acid is used to stop the reaction producing a yellow color
- Optical Density (OD) is read on a plate reader at 450 nm
- OD is inversely proportional to the amount of cortisol present in specimen
- A standard curve converts OD to concentration/volume (ug/dL)
Enzyme Immunoassay (EIA)  
Competitive Binding

1. Wells are coated with antibodies.
2. Standards & unknown samples are added.
3. An analyte linked to an enzyme (cortisol peroxidase) is added.
4. Unbound analytes are washed away.
5. Standards & unknown samples compete w/analyte-enzyme for binding sites.
6. Analyte-enzymes binds with TMB. = Blue Color
7. TMB (substrate) is added.
8. Optical density is read on a plate reader.
9. Stop solution is added. = Yellow Color
Enzyme Immunoassay (EIA)
Competitive Binding

Unbound analytes are washed away

Bound analytes

Conjugate

Standard and/or sample are added

Antibody on EIA plate
Enzyme Immunoassay (EIA)
Competitive Binding

Substrate reaction

Substrate (TMB)

Substrate reaction
Enzyme Immunoassay (EIA)  
Competitive Binding

EIA plate after TMB is added.

EIA plate after Stop Solution is added.
Optical density determined at 450 nm

<table>
<thead>
<tr>
<th>sample</th>
<th>OD</th>
<th>B/Bo</th>
<th>ug/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.09</td>
<td>.048</td>
<td>3.00</td>
</tr>
<tr>
<td>2</td>
<td>.24</td>
<td>.145</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>.52</td>
<td>.340</td>
<td>.33</td>
</tr>
<tr>
<td>4</td>
<td>.90</td>
<td>.593</td>
<td>.11</td>
</tr>
<tr>
<td>5</td>
<td>1.22</td>
<td>.812</td>
<td>.03</td>
</tr>
<tr>
<td>6</td>
<td>1.38</td>
<td>.921</td>
<td>.01</td>
</tr>
<tr>
<td>Bo</td>
<td>1.50</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NSB</td>
<td>.02</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Bo = no cortisol
NSB = Non specific binding (no antibody)

B = (OD – NSB)
Example Standard Curve

4 Parameter Curve

$y = \frac{(A - D)}{(1 + (x/C)^B) + D}$

$A = 0.0111$, $B = -1.0247$, $C = 0.1889$, $D = 0.9752$, $R^2 = 0.9992$
This reaction is inversely proportional:

- High optical density = More color = More analyte-enzyme activity
  
  More analyte-enzyme activity = Less analyte present in unknown samples.

- Less optical density = Less color = Less analyte-enzyme activity
  
  Less analyte-enzyme activity = More analyte present in unknown samples.
Performance Characteristics

- Linearity of Dilution: 91.7%
- Spike Recovery: 100.8%
- Intra-assay Precision: 3.48%
- Inter-assay Precision: 5.08%
- Low Sensitivity Limit: 0.007 ug/dl
- Serum Correlation: $r(47) = 0.91, p < 0.001$
Salivary Alpha Amylase (sAA)

Salivary Enzyme

• Role in Digestion of Carbohydrates and Starches
• Role in Oral Biology
• Surrogate Marker of Sympathetic Nervous System Activation
• Kinetic Reaction assay: Chromogenic substrate (2-chloro-p-nitrophenol linked to maltotriose) used to measure enzyme activity

Test Principle

- Chromagenic substrate used to measure enzymatic activity
- Substrate = 2-chloro-p-nitrophenol linked to maltotriose

\[ \alpha\text{-Amylase} \]

Substrate \[ \rightarrow \] maltotriose + 2-chloro-p-nitrophenol (yellow)

- Measured spectrophotometrically at 405nm
Kinetic Measurements

- A chromogenic substrate is used to measure enzymatic activity.
- Chromogenic substrates are peptides that interact with proteolytic enzymes (which break polypeptide chains into amino acids) under the formation of color.
- After enzyme cleavage occurs a chemical group attached to the peptide part of the substrate is released and produces a color.
- This color change can be followed spectrophotometrically and is proportional to the enzymatic activity.
Kinetic Measurements

• Example of this method is used in our α-Amylase (and Uric Acid) test.

• The chromagenic substrate in this assay is 2-chloro-p-nitrophenol linked with maltotriose.

• The enzymatic action of α-amylase on this substrate yields 2-chloro-p-nitrophenol.
• Plate read at two time points
• Difference of color intensity is linked to enzymatic activity
• α-Amylase activity is directly proportional to increase in absorbance at 405 nm (yellow)
Summary: what we covered

Step 1: Researching Saliva Biomarkers
Step 2: Preparing to Collect Saliva
Step 3: Collecting Saliva (Field or Lab)
Step 4: Organizing, Aliquoting and Storing Samples
Step 5: Transporting Samples for Testing
Step 6: Selecting Assay Kits
Step 7: Testing Samples for Biomarkers and DNA
Step 8: Reviewing Accuracy of Testing Results
Step 9: Preparing Samples for Long-Term Storage
Step 10: Analyzing Saliva Biomarker Data
Publish Findings

Key:
= Salimetrics Products and Services

www.salimetrics.com
Canine saliva collection
sIgA and Acute Stress

Cortisol, Testosterone and the Social Network: Friendship Nominations in Nursing Program

Figure 2. Cortisol and salivary α-amylase (sAA) in response to the combat stressor (M, SE)

Sampling Schemes: EMA

Ecological Momentary Assessment

In context: real world data
Current state vs Trait
Seasonal stress
Monthly, weekly, daily samples
Adolescents: Diurnal alpha-amylase patterns and momentary mood states

Sample and Design

- 50 adolescents (33 females, 28 males)
- 7 diary-saliva sample pairs on each of two typical consecutive weekdays
- Wake, 30 min post wake, bedtime, and 4 random times during the day
- Diary reports of mood states

Findings

- Diurnal rhythm, low in morning and non-linear rise across the day
- Positive associations between sAA levels and intense negative or positive affective status
- In naturalistic everyday social contexts sAA levels reflect concurrent intense affective states and arousal

- ** Recently Replicated with women during pregnancy
Stay connected

Thank you!

The SPIT REPORT  SPITIPS  MY SPIT RESEARCH  MY SPIT LAB  SPIT CAMP