The Stability of Saliva for Hormone Testing: A Pilot Study

There are multiple options in the functional medicine marketplace for the testing of salivary hormone analytes. But how do you know which one is giving you the accuracy and precision your patients deserve? Certainly working with a proven specimen type like saliva is important, but we also need to look at the temperatures required to maintain a quality patient specimen.

Salimetrics (www.salimetrics.com), considered to be the world leader in salivary bioscience research, recommends that all saliva samples be stored promptly at extremely cold temperatures (-20°C) for maximum stability. Although they admit that this is a conservative position (the research standard), they recommend saliva be kept as cold as possible because they’ve observed that the closer to frozen a specimen stays, the better the preservation of the analytes and the more accurate the results.

For example, after several days at ambient temperatures, Salimetrics stability studies have shown DHEA (not DHEA-Sulfate, the more stable form tested by BioHealth) results in a deviation from baseline by 52%. The same ambient exposure conditions for estradiol resulted in a 36% deviation from baseline. Having your patients use test kits that do not require cold storage and expedited shipping is a source of major concern, given how some hormones degrade and cause inaccurate results.

In order to determine the efficacy of different packaging and shipping methods used in functional medicine, BioHealth conducted the following experiment on progesterone and testosterone using real-world shipping conditions. Progesterone is well-known to be unstable unless preserved, while testosterone is reputed to be more stable. Hormones such as cortisol and DHEA-S were omitted from the study, due to their proven stability at ambient temperatures.

As you’ll see BioHealth is the only lab that insists on cold preservation of samples and specific handling steps, to preserve the integrity of sensitive hormone analytes and the subsequent patent test results.

Experiment Model

1. Test kits were obtained from 5 of the major functional medicine labs who perform salivary hormone analysis.

   Shipping and Packaging Methods

   a. Lab 1 (BioHealth): Vials are frozen and shipped overnight on a large cold pack with AM delivery
   b. Lab 2: Vials are stored at ambient temperature and shipped by standard overnight delivery. No ice packs are used.
   c. Lab 3: Vials are stored at ambient temperature and sent by 2-day delivery. No ice packs are used.
   d. Lab 4: Vials are stored at refrigerated temperatures and sent 3-day select. No ice packs are used.
e. Lab 5: Vials are stored at ambient temperature and sent by Ground methods. No ice packs are used.
f. Lab 6: Vials are frozen for at least 2 hours and sent priority overnight. No ice packs are used.

2. Instructions for collection and shipping were followed precisely according to each lab’s patient test instructions. In addition to saliva samples, a USB temperature logger was validated then included in each kit.

3. Kits were sent from the central United States by the method required by each lab, respectively, to a mail forwarding service located near each lab, then sent to BioHealth.

4. The temperature fluctuations each package was subjected to were emulated by programming the time intervals and temperatures recorded by the temperature loggers into a series of digital incubators.

5. 38 volunteers donated saliva specimens which were aliquoted then subjected to each lab’s respective collection and shipping conditions, as well as a sample that was tested immediately to provide a baseline for comparison.

6. After the time interval of the applicable environmental conditions transpired, each sample was tested and compared to the baseline test.

**Temperature Fluctuations during Transit**

We found that most labs’ shipping and handling methods subjected samples to extreme swings in temperature. We believe much of this is due to time in delivery vehicles where climate control is non-existent and inferior packaging does not keep samples cold, and in many cases, not even cool. Observed temperatures, from other labs, ranged from a low of 48°F to a high of 109°F. Using the data loggers, we observed multiple rises and falls in samples temperature between time of shipping and arrival at our lab. We believe these swings are a major contributing factor to compromised stability.

In contrast, when we looked at the temperatures recording during the travel of a BioHealth test kit we found great consistency. During the journey, the temperature inside the kit stayed between 35°F and 41°F degrees Fahrenheit.

It is also important to note that only BioHealth requires the patient to freeze their samples before shipping. The process of freezing saliva as soon as possible serves to inhibit bacterial growth and also precipitates mucins in the sample, in order to improve the pipetting procedure and accuracy at the lab.

**Summary of Results**

**Progesterone**

Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis. It has broad reaching effects and is a critical measurement in mapping the female cycle, determining dosages of HRT and understanding the cause of many female hormone related dysfunctions.
While BioHealth’s shipping methods resulted in an average of 88% recovery of baseline results, other labs’ shipping and handling methods did not fare as well.

Lab 1 (BioHealth Lab): 88% recovery to baseline samples
Lab 2: An average of 50% recovery of baseline samples
Lab 3: An average of 37% recovery of baseline samples
Lab 4: An average of 55% recovery of baseline samples
Lab 5: An average of 48% recovery of baseline samples
Lab 6: An average of 70% recovery of baseline samples

**Testosterone**

Because salivary testosterone has a more specific, narrow testing range for its quality control standard solutions, large changes in measurement correspond to significant deterioration of the sample. Therefore, it is best to examine testosterone in the context of the clinically significant percentage change from baseline result in order to see the degradation of samples during transit.

In many salivary hormone stability studies the standard is to consider any result that isn’t within 15% of the baseline to be a failure. For every 10 samples, 8 (80%) should be within 15% of baseline to be considered stable. This criterion puts patient safety first, emphasizing concise data for interpretation and possible treatments.

What we saw in our experiment is that only BioHealth’s packing and shipping methods led to a result that would be considered a success.
Lab 1 (BioHealth): 81% of all samples within 15% of baseline samples
Lab 2: 39% of all samples within 15% of baseline samples
Lab 3: 42% of all samples within 15% of baseline samples
Lab 4: 58% of all samples within 15% of baseline samples
Lab 5: 61% of all samples within 15% of baseline samples
Lab 6: 58% of all samples within 15% of baseline samples

### Testosterone
Percentage of Samples within 15% of Baseline Value

<table>
<thead>
<tr>
<th>Lab</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab 1 (BioHealth) (35-41°F)</td>
<td>81%</td>
</tr>
<tr>
<td>Lab 2 (70-93°F)</td>
<td>39%</td>
</tr>
<tr>
<td>Lab 3 (70-109°F)</td>
<td>42%</td>
</tr>
<tr>
<td>Lab 4 (66-97°F)</td>
<td>58%</td>
</tr>
<tr>
<td>Lab 5 (64-102°F)</td>
<td>51%</td>
</tr>
<tr>
<td>Lab 6 (46-81°F)</td>
<td>58%</td>
</tr>
</tbody>
</table>

**Final Words**

Sending progesterone and testosterone by any method in which samples were not required to be frozen before being shipped overnight at refrigerated temperatures resulted in discrepancies that could result in inaccurate test results and misguided decisions for interpretation and treatment. While this study was fairly small, we believe it is an accurate representation of the results of erratic and poorly controlled transit variables on progesterone and testosterone. There analytes that need to be shipped as cold as possible to ensure accuracy. In the future, BioHealth will be running similar tests and expanding the number of analytes tested. In the meantime, our commitment to putting discernable accuracy above shipping costs will remain unchanged.