



## Saliva as a Possible Second Sample Matrix

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### Abstract:

Since 1985, the state of New Hampshire (NH) has captured breath samples for reanalysis by defendants in Driving Under the Influence of Alcohol cases. NH collects breath samples on silica gel using the Intoxilyzer 5000EN. Because breath capturing is a blind action and can be influenced by many unseen elements, we are investigating a better representation for second sample analysis.

Saliva is a mixed matrix capable of being used in multiple testing platforms ranging from biological protein markers and disease vector identifications to drugs and alcohol detection. It has been documented that saliva alcohol concentrations (SAC) reflected blood and breath alcohol concentrations (BrAC) when direct samples of saliva were tested. For this initial study we used two versions of the “Quantisal™”, saliva collection device with a volume adequacy indicator” to collect our saliva samples.

Sixty-seven paired saliva and breath samples were collected and analyzed. The breath samples were analyzed on an Intoxilyzer 5000EN before the saliva collection as well as having a 0.100g/210L external reference run post each test. The first set of saliva samples were collected on a version of the Quantisal™ device that contained a crease in the collection pad while the second set of samples were collected on a modified Quantisal™ device without a crease in the collection pad. All the saliva samples were analyzed by heated headspace gas chromatography with a flame ionization detector (HSGC-FID).

The initial analytical results produced 1.35 to 1 saliva to breath correlation. Also an initial stability study involved reanalyzing saliva samples at 10 days and 1 month post collection (samples stored at 4°C). A decrease in the SAC reached an average of 38% for the samples stored for 10 days while the samples stored for one month were depleted of detectable amounts of alcohol.

### Introduction:

Since 1990 the State of NH Breath Alcohol program has been using silica gels to capture breath samples on Intoxilyzer 5000s. We have been through three generations of Intoxilyzer 5000s and at present are using the 5000EN. Prior to 1990 we used a different instrument and a different collection material. In all cases we have battled the blind sample capture for many years. The operator has no way of knowing if the unit collected the samples properly. If there is a valve failure or the operator inadvertently leaves the mouthpiece on, the captured samples can be extremely higher without any visual indication to alert the operator.

The correlation of saliva alcohol concentrations (SAC) to blood alcohol concentrations (BAC) have been reported as 1.21:1 (Linde '32), 1.08:1 (Jones '79) and 1.02:1 (Gubala & Zuba '02). The last report used 1,152 samples and had  $R^2$  of 0.94. Jones has also report the correlation of saliva to breath is slightly better the saliva to blood.

### Methodology:

At the controlled drinking sessions the subjects (four men and eight females) after baseline testing were allowed to drink whatever alcohol containing beverage they choose ranging from beer to wine to mixed drinks. 15 minutes after each drink, a breath test was taken on an Intoxilyzer 5000EN followed by a 0.100 g/210L external standard to monitor for any drift.

We used the “Quantisal<sup>™</sup>, saliva collection device with a volume adequacy indicator” version one in the first session. This device had a small slit in the top of the collection pad just below the handle containing the indicator window. The subjects placed the device in their mouths between cheek and gum for 2 to 10 minutes until the indicator window was totally blue. This indicates 1ml of oral fluid has been collected.

During sessions 2 and 3 we used the second version of the Quantisal<sup>™</sup> collection device which did not have the slit in the collection pad. Otherwise the procedure was the same. 77 paired saliva/breath samples were collected; however, 10 saliva samples could not be used because the collection device separated leaving a much shorter path to the indicator. This failure caused the indicator to turn blue prematurely on a suspected short collection. In these cases we could not tell if enough saliva had been collected and therefore discarded those samples.

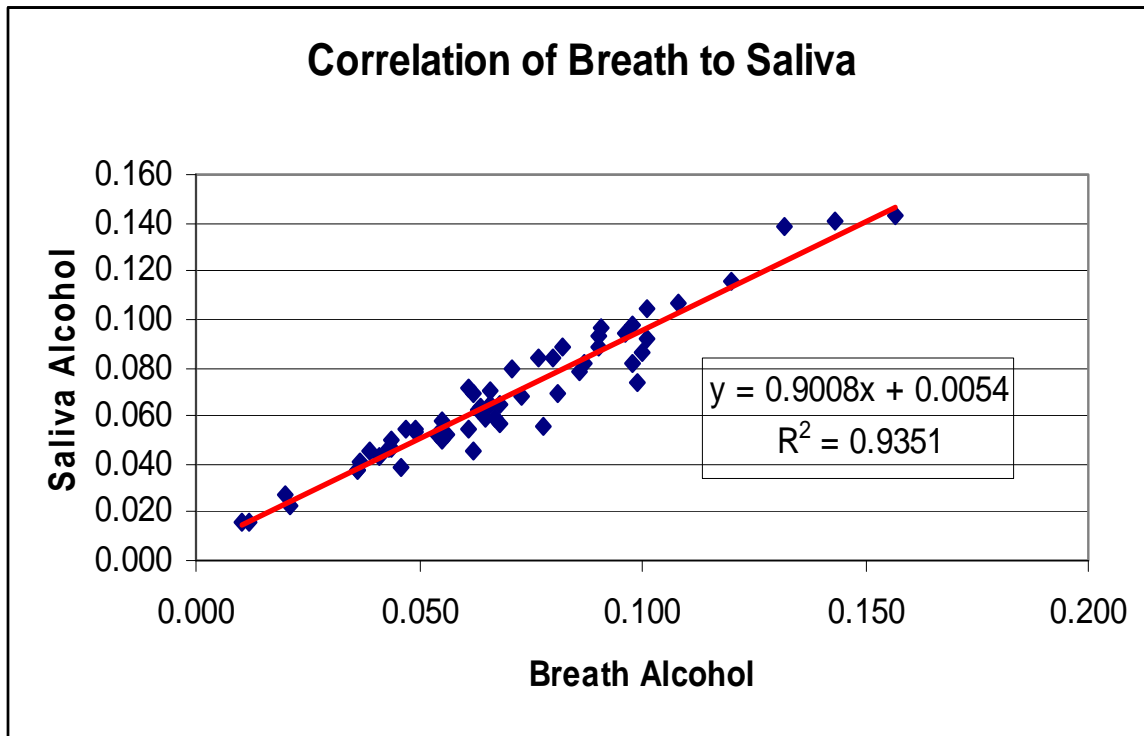
The saliva samples were analyzed on a heated headspace gas chromatograph with flame ionization detector within one week of collection. The standard operating procedure used for the saliva analysis followed the same protocol used in our blood alcohol analysis with n-propanol as the internal standard. The samples were stored at 4°C before and after analyses.

A small portion of the highest alcohol containing saliva samples from the second and third sessions were reanalyzed at 10 days and one month post collection.

### Results:

When the saliva values were plotted against the breath values, the linear regression produced a correlation ( $R^2$ ) value of 0.9351 (see figure 1).

Figure 1



**Discussion:**

Initial results indicated a good correlation exists between the SAC and BrAC. Thus far, the t value for saliva / breath is 1.35. However, this may change as our method for analysis undergoes additional revisions including a potential modification in the saliva capturing device minimizing failed captures. For example, several practical issues developed that may have affected the results if not handled properly. One, the collection device itself even without the slit tends to tear sometimes. This could lead to a choking situation if the pad is swallowed by the subject. Two, the indicator must be carefully watched and allowed to completely fill the window. It does not seem possible to over saturate the paddle but short samples definitely affect the results. Three, the samples are diluted 1:3 so at low alcohol concentrations the gas chromatograph limit of detection may be reached. This may have lead to some correlation issues. Four, there is no preservative in the diluents. We found the alcohol concentrations decreased markedly (38%) after 10 days of storage and after 1 month of storage, alcohol was not detected.

#### Conclusions:

Data indicates saliva alcohol concentrations (SAC) correlate well with the breath alcohol concentrations (BrAC). Saliva samples provide is a viable matrix for alcohol testing. They are non invasive and relatively simple to collect. Thus, in alcohol related cases, law enforcement has the ability to directly collect samples minimizing the need for medical personnel. We continue to investigate sample handling and storage conditions as they relate to sample stability.

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