Summary:

Several studies have documented the close correlation between alcohol levels in saliva and whole blood. A new method has been developed for the rapid, quantitative determination of ethanol in saliva, the STATlab™ Saliva Alcohol Test. Clinical Trials compared saliva alcohol levels measured by STATlab™ to blood levels measured by gas chromatography and serum measured by another enzymatic assay. The correlation of saliva levels to blood and serum were $r=0.98$ and 0.97, respectively. The study has shown that saliva alcohol determinations can be an accurate measure of intoxication.

Introduction:

Non-invasive alternatives to blood sampling for ethanol analysis have long been sought. Venous whole blood is the "gold standard" specimen for ethanol analysis. Serum or plasma levels correlate well with whole blood ($r=0.995$), but blood products must be drawn in a clinical setting by a trained professional. Ethanol levels are 1.2 times higher in plasma or serum than in whole blood due to the higher water content of the cell free fluids (Payne, et. al., 1968). Some of the alternative samples which have been evaluated include breath, tears, urine and saliva. A good review of this literature is found in Caplan (1987).

Breath is the easiest and cleanest sample to collect but does not correlate well with venous blood over time. Breath analysis is a measure of alcohol in arterial blood and during the absorptive period, venous blood and arterial blood ethanol values can be dramatically different (Widmark, 1981). A partition ratio is used to compare breath to blood. The standard ratio is 2100, but independent studies have shown that this ratio can vary from 900 to 3100 in different individuals (Mason and Dubowski, 1976).

Because urine is the standard test solution for drugs of abuse screening, alcohol testing is frequently done simultaneously. However, ethanol metabolism varies extensively from person to person. Ethanol also acts as a diuretic and the ethanol dilutions vary in different individuals depending on the degree of diuresis. The reported ratios in individual studies for urine to blood ethanol are very wide, anywhere from less than 1 to greater than 2 (Caplan, 1987).

Tears have also been studied as an alternative sample, but sample collection is difficult. The correlation between tears and blood is better than urine but still not very good. See Caplan (1987) for specific data.

Saliva has also been compared to blood for ethanol analysis. Jones reports blood to saliva correlation of $r=0.976$ (1979). Ratios reported in published studies range from 1 to 1.3 (Caplan). Despite the good correlation between saliva and blood there has been no convenient method to collect, handle and test saliva for alcohol determination.
The STATlab™ Saliva Alcohol Test has recently been developed for the quantitative determination of ethanol concentration in saliva in a simple-to-use format.

Materials and Methods:

The STATlab™ Saliva Alcohol Test was used to measure ethanol concentrations in saliva. The STATlab test is a disposable enzymatic assay device which uses a proprietary color development technology. The test is an endpoint measurement; actual ethanol concentrations are measured by keeping the sample volume small (45ul) without need for user measurement. This test is unique in that the output is a colored bar the length of which is directly proportional to the blood ethanol level. The bar develops within two minutes and is stable for in excess of 15 minutes.

The enzymatic reaction uses alcohol dehydrogenase and diaphorase. Figure 1 shows the enzyme reactions. The electron sink is part of the proprietary color control system. For a more complete description of STATlab see Timmerman, et.al. (1989).

Clinical Studies:

Forty-two healthy subjects with no prior history of alcoholism were given 4.5-7.5 ounces of alcohol over 90 minutes. The alcohol was given in the form of liquor, wine or beer. Blood and saliva samples were taken at 30, 60, 90 and 120 minutes following the last drink. The blood samples were sent to a local clinical laboratory for analysis by gas chromatography. Saliva samples were collected and analyzed with the STATlab test as demonstrated in Figure 2.

In a subsequent trial, twelve healthy subjects followed the same alcohol consumption protocol. In this trial, serum samples were analyzed using an enzymatic method and saliva samples were analyzed by STATlab.

Results:

Excellent agreement was obtained between the saliva and the blood samples. Figure 3 shows the comparison of saliva by STATlab versus whole blood by gas chromatography. The correlation coefficient of this line is 0.977 and the slope of the line is one. Figure 4 is the presentation of the 95% confidence levels of this data. In all cases the saliva samples would be essentially identical to the blood samples. The correlation between serum and saliva, both measured enzymatically, was also excellent. The correlation coefficient was 0.967 and the slope was 0.83. The literature does not report serum to saliva correlations or ratios but does report serum to whole blood values. Since the ratio of serum to whole blood is 0.8, and the ratio of serum to saliva is 1, one would predict that the ratio of serum to saliva would also be 0.8. Figure 5 presents this set of data.

Discussion:

Saliva can be a difficult material to collect. The cotton tipped collector supplied with the STATlab device effectively filters out various debris from the saliva, leaving a sample which appeared to be homogenous to be injected into the device. It was essential, however, that the cotton tip be saturated
saliva samples, but larger sample volume usually solved this problem.

In a few cases the STATlab device was not completely filled with saliva. The STATlab instructions require that the user check the QA Spot™ at the end of the device for color. This built-in quality assurance indicator serves two functions: it verifies that the enzymes are functioning and it indicates that the whole reaction capillary has been filled with saliva. This proved to be a very valuable tool as there were a few tests which were repeated due to insufficient filling of the device. No data was lost due to non-filling of devices since the QA feedback signaled the tester to immediately collect another sample. The number of incompletely filled devices decreased as the users became more familiar with the device; 9 devices were repeated because a QA Spot did not develop.

Saliva analysis was much simpler than the blood assay. The subjects collected their own saliva samples and presented the applicators to the designated testers. Results were available within 2 minutes and were very easy to read; it was very easy to distinguish between the end of the blue bar and the yellow background. Most subjects waited for the test to develop and appreciated the immediate feedback on their ethanol level.

Conclusion:

Previous work has shown that excellent correlations exist between blood, plasma and saliva ethanol levels. The current study demonstrates that saliva alcohol determinations can be an accurate measurement of intoxication. An accurate, disposable, rapid test for measuring ethanol concentration in saliva is now available and may simplify testing.

REFERENCES


