Saliva Alcohol

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Physiology of Saliva
Excreted by Parotid, SM, and SL Glands

0.5 - 1.5 L/day
serous and mucous alveoli
99% water w 0.3% protein and 0.3% mucins
ave pH 6.4 range 5.6-7 unstimulated
ave pH 7.0 max 8.0 stimulated

Submandibular gland 65%
Parotid gland 23%
Sublingual 4%
Alcohol Saliva/Blood Ratio

- Friedemann et al 1938
- Newman Abramson 1942
- McColl 1979
- Jones 1979
- Haeckel 1987
- Jones 1993
- Bendtsen et al 1999

- close correlation
- close agreement
- 1.05 parotid, 0.95 mixed saliva
- 1.082 (s.e.m 0.0059)
- S/P = 0.85
- 1.094 (range 0.88-1.36)
Fig. 3. Scatter plots and regression statistics for saliva alcohol vs blood alcohol (top), and breath alcohol vs blood alcohol (bottom).

- N = number of correlates;
- r = correlation coefficient;
- $s_{yx}$ = standard error estimate (given as a percentage of $\bar{y}$).
Fig. 2. Mean concentration–time profiles of ethanol in capillary blood end-expired breath, and mixed salivary secretions.

The traces are mean (± SE) for 21 healthy men who drank ethanol 0.68 g/kg body weight, in 20 min after an overnight fast.
Before Saliva EtOH Test

☐ Wait 10 minutes to allow mouth to clear after eating or drinking before collecting saliva. Failure to comply may result in an invalid test reading.
DOT Conforming Products List
Saliva Alcohol Screening Tests
www.nhtsa.dot.gov/people/injury/alcohol/blood

- Roche Diagnostics
- OraSure Technologies, Inc. (formerly STC)
- Chematics, Inc.
- On-Site Alcohol®
- Q.E.D. A150 Saliva Alcohol Test
- ALCO-SCREEN 02™
Q.E.D. Saliva Alcohol Test
Conclusions

• Advantages
  – Non-invasive collection
  – Difficult to adulterate
  – On site testing
  – Correlates with BAC and impairment

• Disadvantages
  – Dry mouth may not produce sufficient sample
  – Small sample of sticky fluid
  – Second sample or test needed for confirmation
Saliva Alcohol

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Introduction

Saliva is the secretion product of the saliva glands of the head and mouth. However, the fluids found in the oral cavity are a mixture of predominately saliva, with lesser amounts of gingival crevicular fluid, cellular debris and blood. For this reason the New York Academy of Sciences meeting on saliva testing in 1993 agreed to use the word saliva for glandular secretions which are collected directly from the saliva glands (most often the parotid glands), and oral fluid for fluid collected by placing absorbants in the oral cavity or by expectoration (Malamud 1993).

The advantages of saliva drug testing are mainly twofold. First, saliva alcohol concentrations can be related to blood alcohol concentrations and pharmacological effects. Second, saliva or oral fluid collection is non-invasive, simple and can be done on-site under observation.

Oral fluid collection can be carried out by having the specimen donor himself or herself place a cotton swab or absorptive material attached to a stick into his/her mouth for a few minutes. The oral fluid absorbed on the material is then processed for testing. Saliva can be collected at the site of the incident in accident or crime investigation. If necessary, saliva flow can be stimulated with citrate hard candy or citrate salts or by chewing on gum or rubber to ensure adequate sample volume. Unlike breath testing saliva collection does not require a cooperative effort on the part of the donor. Saliva collection may be an alternative when the person is unable to blow into an alcohol breath instrument for sufficient time or volume. Because saliva collection is non-invasive and can be done by the donor themselves in most situations, it is more acceptable to most persons than providing urine, blood or hair and can be done while the donor is under observation by the collector. An exception may be when the donor is unconscious or so sedated to be unable to follow instructions. Finally, it is difficult to adulterate or substitute oral fluid specimens in an attempt to avoid detection as any adulterating substances held in the mouth will dissipate, be swallowed or spit out during the ten to twenty minute observation period before collection of the specimen.

Like any biofluid from human subjects, oral fluid may transmit infectious agents and should be handled with the appropriate universal precautions for human biological fluids. Saliva contains mucopolysaccharides and mucoproteins which make it less fluid and less easily poured or pipetted than urine. Some drugs, medical conditions or anxiety can inhibit saliva secretion and cause dry mouth.

Anatomy and Physiology of Saliva: The Saliva Glands
The human saliva glands produce between 0.5 and 1.5 liters of saliva daily. During resting conditions most mixed saliva is supplied by the submandibular glands (70%) with lesser amount (25%) from the parotid glands and the remaining (5%) from the sublingual and other minor glands. During stimulation the parotid saliva output increases to about half of the total. Saliva is composed of 99% water 0.3% protein (largely amylase) and 0.3% mucins. The parotid gland produces mostly serous fluid. The submandibular and sublingual glands excrete both serous fluid and mucins. The saliva glands like the liver, kidney and brain, are well supplied with arterial blood.

Salivary glands are composed of two regions, the acinar region which contains the cells which are capable of secretion and the ductal region lined with water-impermeable cells which carry the secretions to the outlets in the mouth. Similarly, saliva formation occurs in two steps. Water and exocrine proteins are secreted by the secretory cells in the acinar region. The fluid which collected in the acinar lumen is isotonic with plasma. As the fluid travels down the saliva ducts, sodium and chloride are reabsorbed while potassium and bicarbonate are secreted. Therefore, when saliva moves rapidly through the ducts, less time is available for sodium reabsorption and the pH of the saliva is higher (Dawes 1964). When the fluid reaches the mouth it is hypotonic to plasma.

Salivary glands are activated by the autonomic nerves. Generally sympathetic stimulation via norepinephrine causes low levels of fluid and high concentrations of protein while parasympathetic stimulation via acetylcholine induces large amounts of fluid secretion.

**Saliva Alcohol Pharmacology**

Ethanol is a low molecular weight compound which passes through cell membranes, does not ionize nor bind to plasma proteins. Ethanol distributes to all body fluids in proportion to their water content. The measured saliva plasma ratio for alcohol, 1.10, is slightly higher than the calculated value, perhaps because of the high blood flow to the salivary glands, saliva ethanol is in equilibrium with arterial blood rather than venous blood collected for analysis. Saliva equilibrates rapidly with blood ethanol.

The passage of ethanol into saliva and the close correlation between saliva and blood alcohol concentrations was reported in the 1930's (Friedemann et al 1938). Jones (1979) reported ethanol saliva/plasma ratio of 1.077 with a range of 0.84 to 1.36 in 48 male subjects between one and three hours after ingestion of 0.72 g/kg ethanol in a fasting condition. Variation was determined by analysis to be equally due to inter- and intra-individual components. Individual variation in saliva/plasma ratios showed no systematic variation through absorption, distribution and elimination phases of ethanol metabolism. Jones 1993 confirmed this value with a measured saliva/plasma ratio of 1.094 (range 0.88 to 1.36) in twenty-one male volunteers. McColl et al 1979 found a highly significant linear correlation between blood ethanol concentrations and those in mixed saliva obtained before and after rinsing and drying the mouth and parotid saliva. McColl pointed out that this only applies if the saliva ethanol is determined in saliva obtained more than 20 minutes after ingestion of ethanol.
Haeckel (1987) reported that ethanol concentrations reached higher peak concentrations in saliva than in peripheral blood. They noted that saliva ethanol concentrations like breath ethanol concentrations more closely correlate with capillary blood than venous blood. Haeckel (1992) reported a saliva/plasma ratio of 0.85 (if related to the aqueous compartment of blood) in saliva and breath samples taken from traffic drivers retained by the police.

Newman and Abramson 1942 correlated saliva alcohol concentrations with ethanol effects on performance. Jones 1993 compared saliva, breath and blood concentration-time profiles with subjective feelings of intoxication, body sway, hand tremor, positional nystagmus and roving ocular movement after 0.68 g/kg ethanol ingestion by fasting male subjects. Saliva concentrations were higher than blood and breath concentrations. All three correlated equally well with measures of alcohol effects. Maximum impairment was reached at the same time as peak saliva, blood and breath levels. The mean elimination rate of ethanol from saliva of 130 ± 25 mg/L/hr was not significantly different from that from blood, 120 ± 11 mg/L/hr and paralleled the recovery of baseline function in the physiological tests.

Analysis

Ethanol can be analyzed in saliva by the same headspace chromatographic (Jones 1978) or enzymatic methods (Jones 1979) as used for blood. Dipstick or reagent strip tests for alcohol have been reported (Tu et al 1992, Pate 1993) but were found to be too unreliable for use in determining blood alcohol content (Lutz et al 1993, Pate et al 1993, Wong 2002). Current enzymatic tests have proven more reliable as quantitative tests (Christopher 1992, Jones 1995, Smolle et al 1999, Bendtsen et al 1999) and several commercial tests for on-site or point of collection testing of saliva alcohol are available.

An example of a point of collection quantitative test is the Q.E.D. Saliva Alcohol Test (OraSure, Bethlehem, PA, USA). The saliva or oral fluid is collected by the donor with a cotton swab which is applied to the test pad. As the saliva moves along the reagent bar by capillary action any ethanol present is oxidized by alcohol dehydrogenase to acetaldehyde with simultaneous reduction of nicotinamide-adenine dinucleotide (NAD). This results in a cascade of electron donor-acceptor reactions catalyzed by diaphorase, involving FeCN and a tetrazolium salt which proceed to production of a purple-colored endpoint. The length of the resulting purple-colored bar on the Q.E.D. device is directly proportional to the concentration of ethanol in the specimen. The alcohol concentration can be read directly from the height of the colored reaction bar from a printed scale in mg/dL or mg% ethanol just as in reading a thermometer. To get an accurate reading the capillary must draw saliva all the way to the top of the device. This is signaled by development of a purple color within five minutes at the QA Spot at the top of the "thermometer."

Since the saliva moves along the reagent bar and reacts directly with the indicator chemicals, any oxidant in the saliva can cause a false positive. The most common
oxidant found in saliva is ascorbic acid commonly added to fruit juices, sodas and soft
drinks as a preservative. Ascorbic acid is absorbed in the gums and is still found in the
mouth in amounts sufficient to give a false positive with the Q.E.D. Saliva Alcohol test
for up to ten minutes after drinking some soft drinks and sodas. For this reason a 15
minute waiting period before testing after taking anything by mouth is doubly
recommended for saliva alcohol testing with the Q.E.D.

The Q.E.D. Saliva Alcohol test comes in two ranges, 0 to 150 which can be read from
0.01 to 0.15 g/dL, and 0 to 350 which can be read from 0.02 to 0.35 g/dL. The first,
lower, range is for DOT and DUI applications, the second is for hospital and overdose
applications.

An example of a headspace enzymatic assay is the On-Site Alcohol Assay for qualitative
detection of alcohol in urine and saliva (Roche Diagnostics, Nutley, NJ, USA; Varian
Inc. Consumable Products, Lake Forest, CA, USA). The On-Site Saliva Alcohol test is
similar to the Q.E.D. Saliva Alcohol test in that saliva is collected by the donor from
his/her own mouth with a cotton swab and then the swab is applied to the specimen well.
Since alcohols are volatile, alcohol vapors will diffuse from the sample pad to the
reaction pad where they react with alcohol dehydrogenase and diaphorase. The hydrogen
released is transferred to tetrazolium salt to produce a highly colored formazen dye. The
presence of alcohol is indicated by the appearance of a purple plus sign (+) in the result
pad. The On-Site Saliva Alcohol test does not have a control spot so an external control
must be run in each testing session on an additional test unit. Since the test detects
alcohol vapors from the saliva, the saliva sample does not come into contact with the
reagents, so there is no possibility of false positives from oxidizing agents such as
ascorbic acid in the saliva. However the result is qualitative only. The cutoff is 0.02 g%,
so a purple plus sign (+) indicates the presence of alcohol at greater than 0.02 g%.

**Interpretation**

Saliva ethanol concentrations are an accurate reflection of blood alcohol concentrations
Jones (1979, 1993). Saliva alcohol can be used to estimate blood alcohol concentrations,
to estimate pharmacokinetics of ethanol in an individual, as evidence of impairment and
to determine fitness for duty in the workplace.

In the USA workplace testing, the Department of Transportation has codified saliva
ethanol testing (DOT 1994). The screening cutoff is 0.02 g% saliva or 0.02 g/210 L of
breath. Because the saliva alcohol tests are non-specific chemical screening tests which
may react with oxidizing agents other than alcohol and with ketones and alcohols other
than ethanol, it is necessary to confirm any positive results greater than 0.02 g% with an
independent test based on a different chemical principle. Breath alcohol tests are usually
either fuel cells or infrared spectrophotometers with optical filters and computer software
safeguards for specificity and sensitivity. Specifically they must be able to distinguish
acetone from ethanol at the 0.02 g% alcohol level. A time limit is put on confirmation of
saliva ethanol results since ethanol is rapidly metabolized by the liver. Initially the
confirmation breath test had to be performed within 20 minutes. DOT now requires the confirmation test to be done within 30 minutes.

DOT 1994 requires a 15 minute waiting period before saliva or breath alcohol tests. DOT describes this as a safeguard against false high readings due to accumulation of mouth alcohol, but the 15 waiting period will also protect against false positive readings from food or drink components such as ascorbic acid which might cause false positive readings in saliva tests. To obtain a correct test, the most important factor is that a waiting period be observed in which the donor does not eat, drink, smoke or belch (that is, nothing by mouth).

References


