U.S. Department
of Transportation
**National Highway
Traffic Safety
Administration**

DOT HS 807 893
Technical Report

December 1992

Laboratory Testing of a Saliva-Alcohol Test Device by Enzymatics, Inc.

This publication is distributed by the U. S. Department of Transportation, National Highway Traffic Safety Administration, in the interest of information exchange. It documents a technical evaluation of the laboratory performance of the Q.E.D. (A150) Saliva Alcohol Test manufactured by Enzymatics, Inc. of Horsham, Pennsylvania. The United States Government assumes no liability for the report's contents or use. Mention of the manufacturer's name and the Q.E.D. product name are only made because they are considered essential to the object of the publication. This report should not be construed as an endorsement of the product or a recommendation for its use. The United States Government does not endorse products or manufacturers.

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT HS 807 893		

4. Title and Subtitle	5. Report Date
Laboratory Testing of a Saliva-Alcohol Test Device by Enzymatics, Inc.	December 1992
	6. Performing Organization Code: DTS-75
	8. Performing Organization Report No.
7. Author(s): Flores, Arthur L., Spicer, Arnold and Frank, James F.	
9. Performing Organization Name and Address: Transportation Systems Center, Kendall Square, Cambridge, MA 02142	10. Work Unit No. (TRAIS)
	11. Contract or Grant No.: PPA No. 243
	13. Type of Report and Period Covered: Technical Report
12. Sponsoring Agency Name and Address: National Highway Traffic Safety Administration, Office of Program Development and Evaluation, 400 Seventh Street, S.W., Washington, DC 20590	14. Sponsoring Agency Code

15. Supplementary Notes

16. Abstract

This study examined the accuracy of a new saliva-alcohol test device (Enzymatics, Inc. "Q.E.D.-A150") at nine different BACs under three temperature conditions. However, it did not assess the saliva collection procedures. The findings indicate that this device appears to provide a useful means for estimating blood alcohol concentrations from saliva samples for screening purposes. Furthermore, laboratory performance on these tests consistently underestimated the target BACs at all alcohol concentrations and all temperatures tested. From a police enforcement perspective, these underestimates would minimize the likelihood of false-positive readings. In other words, these results suggest that it is less likely that police would identify someone as having a BAC above a given threshold when the person being tested is, in fact, not above that level. However, it should be remembered that this limited laboratory evaluation does not address any issues related to collection of saliva samples or police field use of the device, such as how much saliva is sufficient for a test, possible health and safety concerns for a suspect or an officer, liability concerns for an agency using the device and police training requirements. Such issues must be considered prior to reaching a conclusion regarding the practical utility and application of the device.

17. Key Words	18. Distribution Statement
alcohol, saliva, saliva-alcohol test device, highway safety, alcohol and highway safety	Document is available to the public from the National Technical Information Service, Springfield, VA 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
unclassified	unclassified		

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

Impairment by alcohol continues to be a significant contributing factor in highway crashes. New technological advances are introduced in the marketplace from time to time to measure alcohol concentration in the body. One such device is a single-use disposable saliva-alcohol test device called the "Q.E.D(A150)"[1] that is primarily intended for use in medical settings. However, the device has potential applications in the highway safety setting. Accordingly, the manufacturer asked the National Highway Traffic Safety Administration (NHTSA) to evaluate it. This report describes the limited laboratory evaluation completed for NHTSA at the Transportation Systems Center (TSC) in Cambridge, Massachusetts.

The Device

The Q.E.D.(A150) Saliva Alcohol Test device is designed to measure saliva alcohol concentration as an estimate of coexisting blood alcohol concentration. Although saliva alcohol concentrations are viewed as essentially the same as the blood alcohol concentrations[2] [3], the manufacturer still recommends that when positive saliva test results occur and exact blood concentrations must be known, the saliva test be followed by direct blood-alcohol measurements. They point out that this more precise blood-alcohol measurement is needed because normal physiological variability in the saliva-alcohol measures still exists at the concentrations of interest.

A single, disposable unit of the device is packaged in a heat-sealed foil pouch. The device is designed for a single use only, after which it is discarded. There are three elements in the foil pouch: a small desiccant package which extends the pre-use shelf life of the device, a saliva swab for obtaining a sample from the mouth, and the device itself. The manufacturer's

---

[1] Introduced by Enzymatics, Inc., 500 Enterprise Road, Horsham, PA 19044.

[2] Jones, A. W., "Inter- and intra-individual variations in the saliva/blood alcohol during ethanol metabolism in man." Journal of Clinical Chemistry, 1979, 25, 1394-1398.

[3] Jones, A. W. "Distribution of ethanol between saliva and blood in man." Clinical and Experimental Physiology, 1979, 6, 53-59.

instructions for collecting a saliva sample tell the user to put the provided cotton swab around the cheeks, gums and under the tongue for 30-60 seconds. The saturated swab is then placed into the entry port of the test device. This report did not assess the adequacy of the saliva collection procedures, but only evaluated the saliva-alcohol measuring technology. The device itself resembles a small plastic room thermometer. It contains a port into which the saliva sample is applied by inserting the swab. Once applied, the saliva is drawn by capillary action through a capillary channel containing a narrow reagent strip.

The reagent strip contains an enzymatic reaction system (alcohol dehydrogenase/nicotinamide-adenine dinucleotide with indicating dye). Reaction with alcohol in the saliva sample causes a portion of the reagent strip to change color. The length of the colored bar so produced, as measured with the adjacent numerical scale from 0.00 to 0.15%, indicates an alcohol concentration in the same way a common thermometer reads temperature. Although not stated on the device, the units on the scale are blood alcohol units and not saliva alcohol units.

Instructions for use of the device are printed on the foil pouch as well as in accompanying product instructions provided by the manufacturer in each box of ten devices. Test results become readable two minutes after sample application.

This test is distinguished from a previously evaluated enzymatic saliva test device[4] in that the user reads a numerical result for this test. The previously evaluated test presented results by a color shade only, which was then compared with a printed color comparator to estimate a BAC.

Test Procedure

Performance of the test devices was examined at three temperature levels and nine (9) BAC levels using spiked alcohol-in-water samples. The manufacturer stated that the device analyzes saliva or water solutions equally well. Known alcohol-in-water solutions were prepared for these tests, because of the handling difficulties and inconvenience when using human saliva samples. Data using human saliva were also collected for a smaller number of tests at five different BAC levels (0.033%, 0.050%, 0.080%, 0.100%, and 0.120% BAC) to confirm the manufacturer's statement regarding the equivalence of alcohol-in-water vs. saliva-alcohol testing.

The temperatures examined were normal room temperature (21° C =

---

[4] Frank, J. F. and Flores, A. L. "Laboratory Testing of Alcoscan saliva-alcohol test strips." Washington, DC: U. S. Department of Transportation, National Highway Traffic Safety Administration, 1986. NHTSA Technical Report No. DOT HS 807 059.

69.8° F.) and high and low values approximating outside winter and summer temperatures (10° C and 35° C, which is 50° F and 95° F respectively). The BAC levels used were: 0.02%, 0.03%, 0.05%, 0.06%, 0.07%, 0.09%, 0.10%, 0.12%, and 0.14%. At room temperature, data on 20 devices at each condition were collected. At the high and low temperature conditions, data on ten devices at each condition were collected. Data on ten devices per condition were collected when comparing the saliva-alcohol vs. alcohol-in-water samples.

Stock alcohol solutions were prepared and appropriate dilutions made to obtain the desired concentrations. These solutions were stored in rubber stoppered 10 cc glass serum bottles from which samples were obtained using the swabs packed in the test pouches. These solutions are stable and were used over a period of several weeks. When tests using spiked saliva samples were run, the saliva was collected and used on the same day.

The tests were performed by following the instructions printed on the pouch. The foil pouch was torn open and the test placed on a flat surface. The swab was dipped into one of the test solutions. The liberally loaded swab was then inserted into the sample port of the device. Successful capture of the sample by the capillary tube is indicated by a change in color of the "QA" spot at the end of the capillary tube. If the "QA" spot fails to change color within two minutes, which rarely was the case, the test was discarded. In a few instances, small gaps were seen within the color-developed part of the capillary tube, and sometimes the end of the color-developed part of the tube was more diffuse (fuzzy) than normal. In these few instances, the estimated end of the colored part of the tube was recorded. The ruled scale on the device allows measurement in terms of blood alcohol concentration (gm/100 ml or mg/100 ml) with the scale running from 0.00 to 0.15% at intervals of 0.01. For each test, best estimates to the third decimal place were recorded.

Results

The performance of the saliva-alcohol test devices under room temperature conditions is presented in Table 1. Tables 2 and 3 present the results under low and high temperature conditions. For each BAC tested, the mean, standard deviation and systematic error are presented at the bottom of the tables. Additionally, regression data are included at the bottom of each table.

As indicated in Table 1, the mean BAC levels obtained from these tests were about 0.007% BAC units (grams/100 milliliters) below the known target value. The average standard deviation obtained is about 0.003%. The 95% confidence interval runs from just below the true target value to -0.006% BAC units below the obtained mean value. This performance falls outside of the tolerance required for placement of evidential breath test instruments on the NHTSA conforming products List. For inclusion

on the NHTSA Conforming Products List, mean values must fall within 0.005% BAC units with a standard deviation not greater than 0.004%. (These last values stated approximately for purposes of comparison).

The data in Tables 2 and 3 for tests conducted at 10° C (50° F.) and 35° C (95° F) respectively, are similar. Performance is slightly better at the low temperature and slightly worse at the high temperature tested.

Table 4 presents data for actual human saliva-alcohol mixtures. Fewer concentration levels were tested because of the slowness of saliva collection and the desirability of using the fluid on the same day of collection. The data obtained are very similar to those obtained in the alcohol-in-water tests, indicating that there are no measurable differences between the two.

Table 5 summarizes the regression data from Tables 1-4.

Conclusion

The Enzymatics, Inc. Q.E.D. A150 Saliva Alcohol Test appears to provide a useful means for estimating blood alcohol concentrations from saliva samples for screening purposes. Furthermore, laboratory performance on these tests consistently underestimated the target BACs at all alcohol concentrations and all temperatures tested. From a police enforcement perspective, these underestimates would minimize the likelihood of false-positive readings. In other words, these results suggest that it is less likely that police would identify someone as having a BAC above a given threshold when the person being tested is, in fact, not above that level. However, it should be remembered that this limited laboratory evaluation does not address any issues related to collection of saliva samples or police field use of the device, such as how much saliva is sufficient for a test, possible health and safety concerns for a suspect or an officer, liability concerns for an agency using the device and police training requirements. Such issues must be considered prior to reaching a conclusion regarding the practical utility and application of the device.

Table 1. Test Results. Enzymatics, Inc. QED A150 Saliva Alcohol Test. Ambient Temperature 21°C (69.8°F).

	Aqueous Alcohol Concentration, gm/100ml								
	.02	.03	.05	.06	.07	.09	.100	.120	.140
1	.014	.021	.045	.053	.065	.085	.093	.114	.138
2	.015	.026	.045	.051	.067	.088	.094	.108	.133
3	.015	.023	.040	.054	.062	.082	.091	.108	.138
4	.015	.021	.044	.052	.064	.082	.095	.112	.139
5	.016	.025	.041	.051	.061	.082	.093	.100	.131
6	.017	.024	.045	.052	.063	.084	.093	.108	.130
7	.016	.021	.042	.051	.062	.084	.092	.107	.124
8	.017	.023	.047	.054	.067	.086	.093	.109	.135
9	.015	.024	.044	.052	.063	.087	.095	.110	.136
10	.016	.022	.048	.051	.062	.083	.091	.105	.130
11	.018	.023	.042	.050	.061	.083	.090	.109	.134
12	.017	.025	.042	.052	.064	.088	.089	.120	.135
13	.016	.032	.044	.052	.062	.070	.091	.109	.126
14	.018	.023	.048	.055	.065	.084	.096	.112	.130
15	.017	.024	.048	.054	.068	.082	.092	.109	.132
16	.017	.024	.047	.055	.065	.084	.096	.120	.137
17	.017	.023	.040	.052	.063	.083	.095	.108	.128
18	.016	.022	.046	.051	.064	.081	.093	.120	.140
19	.016	.021	.040	.079	.061	.083	.090	.115	.131
20	.017	.022	.045	.053	.065	.083	.094	.112	.130
m	.0163	.0230	.0442	.0522	.0637	.0832	.0928	.1108	.1329
sd	.0011	.0015	.0027	.0016	.0021	.0037	.0020	.0051	.0044
se	-3.7	-7.0	-5.8	-7.8	-6.3	-6.8	-7.2	-9.2	-7.1

m=mean, sd=standard deviation, se=systematic error($X10^3$)

Regression Data:
(QED test result =int. + slope x known alcohol conc.)
intercept= -.005, slope=.976, std. err. of est.=.003
corr. coeff.=.993

Table 2. Test Results. Enzymatics, Inc. QED A150 Saliva Alcohol Test. Ambient Temperature 10°C (50°F).

	Aqueous Alcohol Concentration, gm/100ml								
	.02	.03	.05	.06	.07	.09	.10	.12	.14
1	.017	.024	.048	.053	.068	.090	.095	.125	.137
2	.020	.023	.048	.057	.066	.098	.109	.116	.128
3	.016	.023	.047	.054	.069	.090	.104	.123	.139
4	.018	.023	.046	.058	.067	.059	.098	.129	.137
5	.017	.025	.048	.054	.068	.086	.091	.118	.139
6	.016	.021	.047	.054	.068	.085	.092	.123	.135
7	.018	.024	.046	.052	.068	.090	.100	.123	.138
8	.016	.025	.046	.055	.067	.089	.098	.121	.137
9	.018	.023	.048	.053	.065	.089	.098	.126	.138
10	.016	.023	.047	.058	.067	.086	.093	.122	.138
m	.0173	.0235	.0470	.0546	.0676	.0896	.0984	.1223	.1363
sd	.0014	.0013	.0009	.0020	.0009	.0039	.0060	.0040	.0036
se	-2.7	-6.5	-3.0	-5.4	-2.4	-0.4	-1.6	-2.3	-3.7

m=mean, sd=standard deviation, se=systematic error($X10^3$)

Regression Data:
(QED test result =int. + slope x known alcohol conc.)
intercept= -.005, slope=1.04, std. err. of est.=.004
corr. coeff.=.992

Table 3. Test Results. Enzymatics, Inc. QED A150 Saliva Alcohol Test. Ambient Temperature 35°C (95°F).

	Aqueous Alcohol Concentration, gm/100ml								
	.02	.03	.05	.06	.07	.09	.10	.12	.14
1	.014	.023	.040	.053	.056	.077	.088	.108	.128
2	.016	.022	.040	.053	.058	.078	.090	.110	.118
3	.017	.023	.043	.053	.062	.080	.089	.112	.123
4	.015	.023	.044	.051	.060	.080	.091	.105	.114
5	.014	.024	.042	.050	.058	.079	.090	.110	.122
6	.014	.022	.038	.050	.057	.079	.089	.108	.119
7	.015	.020	.038	.050	.061	.083	.090	.106	.128
8	.016	.023	.038	.052	.057	.079	.087	.106	.121
9	.015	.022	.039	.050	.062	.076	.086	.099	.128
10	.017	.023	.041	.053	.070	.079	.091	.113	.133
m	.0151	.0225	.0404	.0515	.0516	.0793	.0893	.1081	.1216
sd	.0011	.0012	.0024	.0014	.0021	.0018	.0013	.0024	.0048
se	-4.9	-7.5	-9.6	-8.5	-18.4	-10.7	-10.7	-11.9	-18.4

m=mean, sd=standard deviation, se=systematic error($X10^3$)

Regression Data:
(QED test result =int. + slope x alcohol conc.)
intercept= -.004, slope=.922, std. err. of est.=.003
corr. coeff.=.992

Table 4. Test Results. Enzymatics, Inc. Saliva Alcohol Test QED A150. Human Saliva Samples.

Saliva Alcohol Concentration, gm/100ml

	.033	.050	.080	.100	.120
1	.030	.048	.076	.099	.115
2	.027	.048	.076	.096	.130
3	.027	.040	.070	.092	.108
4	.026	.044	.075	.044	.108
5	.025	.046	.072	.091	.115
6	.031	.046	.075	.096	.132
7	.030	.047	.074	.095	.108
8	.029	.048	.075	.091	.120
9	.027	.047	.073	.098	.110
10	.026	.048	.073	.097	.118
m	.0278	.0426	.0239	.0949	.1164
sd	.0020	.0025	.0019	.0028	.0088
se	-5.2	-7.4	-6.1	-5.1	-3.6

m=mean, sd=standard deviation, se=systematic error($X10^3$)

Regression Data:
(QED test result =int. + slope x alcohol conc.)
intercept= -.005, slope=1.007, std. err. of est.=.004
corr. coeff.=.982

Table 5. Test Results. Enzymatics, Inc. Saliva Alcohol Test QED A150. Comparison of aqueous and saliva performance. Results calculated from regression data of tables 1-4.

	Alcohol Concentration, gm/100ml			
	.00	.04	.10	SEE
AAC, 21°C	(-.005)*	.034	.093	.003
10°C	(-.005)*	.037	.099	.004
35°C	(-.004)*	.033	.087	.003
Saliva AC	(-.005)*	.035	.096	.004

*QED test readout scale does not extend below zero.
SEE: Standard Error of the Estimate.