BLOOD ALCOHOL TEST
Instructions

EarlyDETECT™
The earlier, the better.

INTENDED USE

The **EarlyDETECT™** Alcohol Rapid Test is intended for the rapid semi-quantitative determination of ethyl alcohol level in human saliva. The test is a rapid enzymatic method to detect the presence of alcohol in saliva for blood alcohol concentration (BAC) greater than 0.02%. It has been published that the concentration of alcohol in saliva is almost equal to that in blood. The tests are designed to obtain a visual, semi-quantitative result and are intended for professional use only. They are neither intended for quantitative results, nor for over-the-counter sale.

The **EarlyDETECT™** Alcohol Rapid Test provides only preliminary analytical data. A more specific, alternative method is required to obtain a confirmed analytical result. To confirm the concentration of positive specimens, an alternate, non-enzymatic technology such as headspace gas chromatography should be used. Clinical considerations and professional judgment should be applied to any test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION OF TEST

Alcohol intoxication can lead to loss of alertness, coma, death and as well as birth defects. The BAC at which a person becomes impaired is variable. The United States Department of Transportation (DOT) has established a BAC of 0.02% (0.02g/dL) as the cut-off level at which an individual is considered positive for the presence of alcohol.

Determination of ethyl alcohol in blood and saliva is commonly used for measuring legal impairment, alcohol poisoning, etc. Gas chromatography techniques and enzymatic methods are commercially available for the determination of ethyl alcohol in human fluids. The **EarlyDETECT™** Alcohol Rapid Test is designed as the screen method to rapidly determine if the BAC level is higher than 0.02% by testing saliva specimen, as set by DOT.

$$\text{EtOH} + \text{TMB} \xrightarrow{\text{ALOx/Peroxidase}} \text{CH}_3\text{CHO} + \text{Colored TMB}$$

PRINCIPLE OF THE PROCEDURE

The **EarlyDETECT™** Alcohol Rapid Test is based on the high specificity of alcohol oxidase (ALOx) for ethyl alcohol in the presence of peroxidase and enzyme substrate such as tetramethylbenzidine (TMB) as shown in the following:

The distinct color on reactive pad could be observed in less than 20 seconds after the tip was contacted with saliva samples with the ethyl alcohol concentration greater than 0.02%. It should be pointed out that other alcohols such as methyl, propanyl and allyl alcohol would develop the similar color on the reactive pad. However, these alcohols are not normally present in saliva.

REAGENTS AND MATERIALS SUPPLIED

- Instruction for use
- Each test contains these materials:

Tetramethylbenzidine (TMB)	0.12mg	Peroxidase(EC)	35 IU
Alcohol oxidase (EC)	0.5 IU	Proteins	0.15mg

MATERIALS REQUIRED BUT NOT PROVIDED

- Saliva sample collection containers
- Timer or clock

WARNINGS AND PRECAUTIONS

- For in vitro use only.
- Do not use a test device beyond the expiration date imprinted on the outside of the foil pouch.
- Avoid cross contamination of saliva samples by using a new sample collection container and pipette for each sample.
- Saliva specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container.
- The product is sensitive to the presence of alcohol and moisture. After opening the package, the test device should be used immediately.

STORAGE AND STABILITY

The test devices supplied can be stored at room temperature or refrigerated (2-28°C) and will remain stable until the expiration date.

Do Not Freeze.

SAMPLE COLLECTION AND PREPARATION

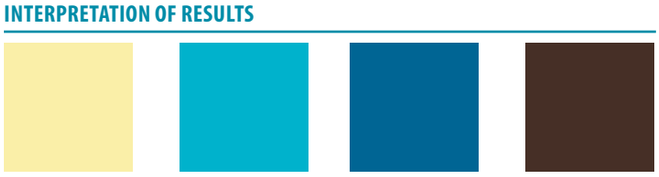
- Nothing should be placed into the mouth of the subject for at least 10 minutes prior to saliva collection. This includes food, drink, tobacco products or other materials.
- Saliva specimen can be collected in a sputum cup or a clean container, or directly applied to the reaction pad of the test strip.
- Avoid contact with skin by wearing gloves and proper laboratory attire.

ASSAY PROCEDURE

1. Do not open foil pouch until ready to begin testing. Refrigerated test device should be allowed to equilibrate to room temperature (15-28°C) before pouch is opened.
2. Remove the test device from the sealed foil pouch by tearing along the notch.
3. Saturate the reactive pad by dipping the reaction pad into the saliva specimen collected in a sputum cup, or by applying saliva directly to the reaction pad. After 10 seconds, shake off the excess saliva.
4. Immediately start timer and at 2 minutes, compare the reactive pad with the provided colored chart.

IMPORTANT: Results after more than 2 minutes may be not accurate. In order to prevent an incorrect reading, do not read the test results after more than 5 minutes. After 5 minutes, the intensity of the colored lines may change. To avoid confusion, discard the test device after interpreting the result.

INTERPRETATION OF RESULTS



Negative: Almost no color change on test pad by comparing with the background of the provided colored chart. The negative result indicates that the concentration of ethyl alcohol in saliva is less than 0.02%.

Positive: A distinct color developed all over the pad. The positive result indicates that the concentration of ethyl alcohol in saliva is 0.02% or higher.

Invalid: The test should be considered invalid if only the edge of the reactive pad turned color that might be ascribed to insufficient sampling. The subject should be re-tested.

PROCESS AND QUALITY CONTROL

Good laboratory Practice recommends the daily use of control material to validate the reliability of device. Commercially available controls that contain sodium azide or other preservatives that will inhibit the enzyme activity cannot be used with this test. The **EarlyDETECT™** Alcohol Rapid Test may be qualitatively verified by using a test solution prepared by adding 10 drops of ethanol alcohol into 8 oz of distilled water. This solution should show a distinct positive result.

AFTER TESTING

Saliva specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container. Residual saliva should be disposed of in a medically approved manner after the completion of all testing, including the confirmatory assay.

PERFORMANCE CHARACTERISTICS

SENSITIVITY: The **EarlyDETECT™** Alcohol Rapid Tests have been designed for detection of alcohol in saliva at the detection sensitivity of 10mg/dL (0.01g/dL), as suggested by DOT. In sensitivity studies performed, samples with concentrations of alcohol equal to or higher than 10mg/dL were identified as positive results for all samples. Thus, the cut-off level of the **EarlyDETECT™** Alcohol Rapid Test was determined to be 10mg/dL.

PRECISION: In order to determine the precision of **EarlyDETECT™** Alcohol Rapid Tests, within-lot and inter-lot reproducibility studies were performed. Results of the within-lot reproducibility studies clearly showed excellent repeatability for all positive and negative saliva samples using one lot of **EarlyDETECT™** Alcohol Rapid Tests. The results of inter-lot reproducibility studies clearly demonstrated that there was no appreciable inter-lot variation when testing both positive and negative samples across three different lots of **EarlyDETECT™** Alcohol Rapid Test.

INTERFERENCE STUDY: The following substances were added to sample which had alcohol levels of 0 and 0.08%. None of the substances at concentration tested interfered in the **EarlyDETECT™** Alcohol Rapid Tests.

The following substances may interfere with the test:

Strong oxidizers	Ascorbic acid	Mercaptans	Bilirubin
Tannic acid	Polyphenolic Compounds	Uric acid	Oxalic acid

These compounds are not normally present in sufficient amount in saliva to interfere with the test. However, the precautious step must be taken so that these materials are not introduced into the mouth during the 10 minute period preceding the test.

Acetaminophen	20 mg/dl
Caffeine	20 mg/dl
Glucose	2,000 mg/dl
Hemoglobin	1 mg/dl
Human Serum Protein	2,000 mg/dl

ASSAY COMPARISONS & EQUIVALENCY

Accuracy and equivalency comparisons of **EarlyDETECT™** Alcohol Rapid Test was evaluated as well as against 86 individual external SAMHSA-certified clinical laboratory samples. The results have been tabulated below.

When compared to the GC data, the relative sensitivity or percent agreement of **EarlyDETECT™** Alcohol Test positive samples with the external clinical study was 41/44 or 93.2%. Negative samples recovered a relative specificity of agreement of 40/42 or 95.2%. The overall relative accuracy obtained was 81/86 or 94.2%.

Alcohol Strip	GC (+)	GC (–)	Row Totals
(+)	41	2	43
(–)	3	40	43
Col. Totals	44	42	86

LIMITATIONS OF PROCEDURE

The **EarlyDETECT™** Alcohol Rapid Test is designed for in vitro detection use with human saliva only. A positive result indicates only the presence of alcohol and does not indicate or measure intoxication.

- There is a possibility that technical or procedure error as well other substances in certain foods and medicines may interfere with the test and cause false results. Please refer to the "Interference" section for a list of substances that will interfere with the test results.
- This diagnostic test provides a semi-quantitative screening for alcohol in saliva. It is not to be used for quantitative determination of alcohol concentration in saliva. To confirm the concentration of positive specimens, an alternate, non-enzymatic technology such as headspace gas chromatography should be used.
- Adulterants, such as bleach or other strong oxidizing agents, may produce erroneous test results when added to saliva specimens, regardless of the analysis method used. If an adulteration is suspected, a fresh saliva specimen should be used.

EXPECTED RESULTS

The **EarlyDETECT™** Alcohol Rapid Test is a semi-quantitative assay. It identifies alcohol in human saliva at a concentration of 0.02% BAC.

BIBLIOGRAPHY

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