MEDICA

IVD CE

**REF 14251-4 4 x 24 mL/10 mL**

**ETHYL ALCOHOL ENZYMATIC (ETOH)**
Wedges each contain usable volumes of 24 mL of R1 reagent and 10 mL of R2 reagent.

**INTENDED USE**
The EasyRA Ethyl Alcohol (ETOH) reagent is intended for the quantitative measurement of ethanol in human urine. Alcohol measurements are used for the diagnosis and treatment of alcohol intoxication and poisoning. The assay is designed for prescription use only on the EasyRA Clinical Chemistry Analyzer. For *in-vitro* diagnostic use only.

**SUMMARY AND EXPLANATION**
Ethyl alcohol can be found in regular alcoholic liquors, in a variety of foods, drinks, candies and in medicinal preparations. When alcohol is ingested, it will quickly spread to whole body and majority (>90%) is metabolize in liver and excreted. After ingestion, alcohol can be found in human urine.

Alcohol intoxication can lead to severe loss of alertness, stupor, coma and death and frequently cause public safety issues. It can also lead to birth defects (fetal alcohol syndrome).[1, 2, 3, 4, 5]

Determination of alcohol concentration is commonly used for determining legal impairment, forensic judgments, diagnosis/treatment of alcohol dependency and detection of alcohol intoxication.

Many different methods are available for the determination of alcohol concentration[1,2] in biological fluid. Medica's Ethyl Alcohol Enzymatic Assay is a two component ready-to-use liquid reagent based on alcohol dehydrogenase (ADH) unique enzymatic reaction. In the presence of nicotinamide adenine dinucleotide (NAD), ADH converts ethyl alcohol to acetaldehyde and reduces NAD to NADH. The ethyl alcohol concentration is directly proportion to the ADH activity. The rate of NADH formation is measured at 340 nm wavelength.

**REAGENTS**

**Buffer Reagent (R1):**
Tris-based buffer (50 mM)
Nicotinamide adenine dinucleotide (NAD, 10mM)

Stabilizers

Sodium azide as preservative

**Enzyme Reagent (R2):**
Alcohol Dehydrogenase(ADH)

Sodium azide (0.09%) as preservative

**Precautions**
1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagent contains less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Material Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

**Instructions for Reagent Handling, Storage and Stability**

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date on the label if stored at 2-8 $^{o}$C. The reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of day programmed on the RFID chip on the reagent wedge. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area.

**SPECIMEN COLLECTION AND STORAGE / STABILITY**
Urine sample may be collected in plastic or glass containers with a tight cap to prevent evaporation. Use fresh urine specimen for the test.If the sample cannot be analyzed immediately, it may be stored refrigerated at 2-8°C for up to 3 days[7]. For longer storage keep sample frozen at -20°C and thaw just before use. Studies have shown that ethanol analytes in urine are stable at -10°C up to 12 months[8]. Samples should be brought to a room temperature of 18-25 $^{o}$C for testing. Samples must be thoroughly mixed before testing. For best results, evaporation caps should be used with all ethyl alcohol samples.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and both samples should be forwarded to the laboratory for testing. Handle all urine specimens as if they are potentially infectious.

Handle all specimens as if they are potentially infectious.

The mandatory guidelines for federal workplace drug testing programs recommends that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units.[6]

**Materials Provided:**
Medica ETOH Reagent Wedge, REF 14251

**Additional materials required:**
Medica EasyCal Ethyl Alcohol Calibrator (Ethyl Alcohol, 100 mg/dL) REF 14696
Medica EasyQC Level A REF 14792
Medica EasyQC Level B REF 14793
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 or
Medica Cleaner Wedge – Chemistry, REF 10661
Medica Evaporation Caps, REF 10745

**Instructions for Use**
The reagent is ready to use as supplied. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray in the reagent area. Dry the neck of the reagent wedge and check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2. Place Medica EasyRA Evaporation Caps, REF 10745 on both the R1 and R2 openings of the reagent wedge.

**NOTE: Use of the Medica EasyRA Evaporation Cap is required to guarantee on-board calibration stability.**

**Calibration**
Medica EasyCal Ethyl Alcohol Calibrator, REF 14696 is recommended for the calibration of the assay. The calibration interval (20 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

**Quality Control**
It is recommended that two levels of human urine-based controls (Low and High) be run with the assay at least once every day and with each reagent lot change. Failure to obtain the proper values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should also follow local, state, and federal quality control guidelines when using quality control materials.

**Results**
Medica Ethyl Alcohol Enzymatic Assay accurately quantifies alcohol concentration in human urine containing 10-500 mg/dL (0.01-0.50%) alcohol.

Ethyl alcohol is not present in detectable concentrations in healthy adults who have not consumed ethyl alcohol. The legal definition of alcohol intoxication varies. Alcohol consumption, metabolism and excretion vary substantially among individuals and dependent upon many factors, for instance, gender, age, weight, time and health conditions…etc (4).

**Procedural Limitations**
1. The legal alcohol intoxication levels vary. The test result should be interpreted in light of clinical signs and symptoms.
2. Ethyl alcohol is volatile. Precaution as suggested in the specimen collection and required to prevent alcohol evaporation from calibrators, controls and samples.
3. The test is designed for use with human urine only.

**PERFORMANCE CHARACTERISTICS**
The results shown below were obtained with the EasyRA analyzer.

**Method Comparison**
Urine specimens containing ethyl alcohol were analyzed on a Cobas Mira with an ethyl alcohol test and on the EasyRA with a Medica ethyl alcohol test. Linear regression analyses of the results are summarized in the following table.

**Medica Ethyl Alcohol Test**

| | **EasyRA vs Cobas Mira**<br>**Urine** |
|---|---|
| **N =** | 75 |
| **Slope** | 0.991 |
| **Intercept** | 0.36 |
| **Correlation** | 0.998 |
| **Range** | 10-464 mg/dL |

**Precision**
The precision of the assay ws measured with drug free human unine spiked with ethyl alcohol following the CLSI guidelines described in EP5-A2 on an EasyRA clinical chemistry analyzer.

| | **Within –run precision (n=80)** | | |
|---|---|---|---|
| | **Mean (mg/dL)** | **SD (mg/dL)** | **%CV** |
| 50 mg/dL | 51.3 | 1.22 | 2.39 |
| 100 mg/dL | 100.7 | 1.32 | 1.32 |
| 200 mg/dL | 197.6 | 2.32 | 1.17 |
| 300 mg/dL | 296.1 | 4.58 | 1.55 |

| | **Total precision (n=80)** | | |
|---|---|---|---|
| | **Mean** | **SD** | **%CV** |
| 50 mg/dL | 51.3 | 1.83 | 3.57 |
| 100 mg/dL | 100.7 | 2.97 | 2.95 |
| 200 mg/dL | 197.6 | 5.02 | 2.54 |
| 300 mg/dL | 296.1 | 7.96 | 2.69 |

**Linearity**
The assay is linear from a concentration 10 mg/dL to 500 mg/dL. A linear regression analysis of linear dilution samples gave the following equation: $y – 0.9989x + 3.65$ ($r^2 = 0.9997$). Samples with an alcohol concentration above 500 mg/dL can be diluted with saline and the results multiplied by an appropriate dilution factor. The reportable range of the assay is 10 to 500 mg/dL. The linear (reportable) range has been established above the Limit of Quantitation for the assay.

**Specificity**
Various compounds structurally similar to ethyl alcohol were tested for cross reactivity. Levels tested exceed toxic concentrations. Therefore, interference is not clinically significant.

| **Compound** | **Level Tested (mg/dL)** | **% Cross Reactivity** |
|---|---|---|
| Acetaldehyde | 2000 | -4.3 |
| Acetone | 2000 | 0 |
| n-Butanol | 2000 | 1.6 |
| Ethylene glycol | 2000 | 0 |
| Isopropanol | 2000 | 0.2 |
| Methanol | 2000 | 0.1 |
| n-Propanol | 2000 | 9.5 |

There is no significant interference (<10%) for the following endogenous compounds:

| | | | | | |
|---|---|---|---|---|---|
| Ascorbic Acid | up to | 1000 mg/dL | Human Albumin | up to | 300 mg/dL |
| Conjugated Bilirubin | up to | 10 mg/dL | Oxalic Acid | up to | 200 mg/dL |
| Total Bilirubin | up to | 15 mg/dL | Sodium Chloride | up to | 2300 mg/dL |
| Creatinine | up to | 500 mg/dL | Gamma Globulin | up to | 500 mg/dL |
| Glucose | up to | 1200 mg/dL | Riboflavin | up to | 7.5 mg/dL |
| Hemoglobin | up to | 100 mg/dL | pH | between 3.0 and 9.0 pH units | |

There is no significant interference (<10%) for the following preservatives up to 1000 mg/dL:

Boric Acid, Sodium Azide, and Sodium Fluoride

**Sensitivity**
At the lower linearity limit of 10.0 mg/dL, the assay exhibits a recovery of +7% and typical precision of 8.1%. The sensitivity of the assay was determined by following the CLSI guidelines described in EP17-4. Using this document, the limit of blank (LOB) was determined to be 0.6 mg/dL, the limit of detection (LOD) was determined to be 1.6 mg/dL, and the limit of quantitation (LOQ) was 7.0 mg/dL. The lower limit of the reportable range is 10 mg/dL.

**REFERENCES**
1 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man. 3rd edition, Chicago, IL. Year Book Medical Publishers Inc.; 1989:322-324.
2 Beutler HO, Ethanol In: Bergmeyer HU, ed. Methods of Enzyme Analysis, Vol VI. 3rd ed., New York: Academic Press, 1984, 598-606.
3 Wyngaarden JB, Smith LH Jr., eds. Cecil Textbook of Medicine. Philadelphia, PA: WB Saunders Co.; 1988: 48-52.
4 Ellenhorn MJ, Barceloux DG, Medical Toxicology. New York, NY: Elsevier Science Publishing Company, Inc. 1988: 525-526, 782-796.
5 Tietz NW, ed. Textbook of Clinical Chemistry. Philadelphia, PA: WB Saunders Co.: 1986: 1704-1706, 1692-1694.

6 Mandatory guidelines for Federal Workplace Drug Testing Program, National Institute of Drug Abuse, Federal Register Vol. 53, No. 69, pp1970 (1988).

7 Hayden, P.M., Layden, M.T., and Hickey, M.D., The stability of alcohol content in samples of blood and urine, Irish Journal of medical science, 146(1):48-53 (1977)

8 Neuteboom, W., and Zweipfenning, P., The stability of the alcohol concentration in urine specimens, *J. Anal. Toxicol.,* 13(3):141-143 (1989)

**EasyRA Parameters:**

| | |
|---|---|
| Primary Wavelength | 340 |
| Secondary Wavelength | N/A |
| Reaction Type | Kinetic |
| Reaction Direction | Increase |
| Reagent Blank | Yes |
| Sample Blank | No |
| Reaction Time | 1.6 Minutes |
| On-Board Stability | 30 Days |
| Cal Stability | 20 Days* |

*with evaporation caps

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