MEDICA

IVD CE

REF 14250-4 4 x 27 mL/9 mL

BUPRENORPHINE-QUALITATIVE (BUP)
Wedges each contain usable volumes of 27 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE
The EasyRA Burprenorphine (BUP) reagent is intended for the qualitative determination of Buprenorphine in human urine at a cutoff value of 10 ng/mL. The assay is designed for prescription use only on the EasyRA Clinical Chemistry Analyzer. For *in-vitro* diagnostic use only.

The assay provides a rapid screening procedure for determining the presence of buprenorphine in urine. The assay provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas Chromatography/mass spectrometry (GC/MS) or Liquid Chromatography/mass spectrometry (LC/MS) are the preferred confirmatory method.[1, 2] Clinical consideration and professional judgment should be exercised to any drug of abuse test result, particularly when the preliminary test result is positive.

SUMMARY AND EXPLANATION
Buprenorphine (BUP) is a semi-synthetic opioid derived from thebaine, and alkaloid of the poppy Papaver somniferum. It is an analgesic often used a substitution treatment for heroin addiction or opiate dependence. Buprenorphine resembles morphine structurally but has both antagonist and agonist properties[3]. Buprenorphine is metabolized in human liver by its N-dealkylation to the pharmacologically active norbuprenorphine, which along with the parent compound is conjugated with glucuronic acid[4,5,6], and excreted in urine. Therapeutically, buprenorphine is as effective as Methadone but exhibits a much lower level of physical dependence, however, study has been shown that buprenorphine has abuse potential and may itself cause dependency.

PRINCIPLE OF THE PROCEDURE
The buprenorphine assay is a homogeneous enzyme immunoassay which provides qualitative results relative to a single calibration cutoff value. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenate (G6PDH) for a fixed amount of antibody in the reagent. Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity.

In the absence of drug in the sample, buprenorphine -labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when free drug is present in the sample, antibody will bind to free drug, and the unbound buprenorphine -labeled G6PDH then exhibits its maximal enzyme activity.

Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance increase that can be measured spectrophotometrically at 340 nm.

REAGENTS

Antibody/Substrate Reagent (R1): Contains monoclonal anti-buprenorphine antibody, glucose-6-phosphate (G6P), Nicotinamide adenine dinucleotide (NAD), stabilizers, sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R2): Contains buprenorphine-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as preservative.

Precautions and Warnings
1. This test is for in-vitro diagnostic use only. Harmful if swallowed.
2. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
3. Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).
4. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
5. 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. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended[8]. 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. For longer storage keep sample frozen at -20°C and thaw then before use. Studies have shown buprenorphine analytes in urine are stable at -20 ^{o}C up to 85 days[9]. Samples should be brought to room temperature of 18-25 ^{o}C for testing. Samples with high turbidity should be centrifuged before analysis.

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.

PROCEDURE
Materials Provided:
Medica BUP Reagent Wedge, REF 14250 (Qualitative)

Additional materials required:
Medica EasyCal Buprenorphine Cutoff Calibrator (Buprenorphine Cutoff, 10 ng/mL), REF 14693
Medica EasyQC Buprenorphine Negative Control (Buprenorphine, 7 ng/mL), REF 14762
Medica EasyQC Buprenorphine Positive Control (Buprenorphine, 13 ng/mL), REF 14767
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 or
Medica Cleaner Wedge – Chemistry, REF 10661
Medica EasyRA 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 Buprenorphine (BUP) Calibrator, REF 14693 is required for the calibration of the qualitative assay. The calibration interval (7days maximum) with Evaporation Caps 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 (positive and negative) 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
The cutoff calibrator, which contains 10 ng/mL of buprenorphine, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance per unit time (mA/min) that is equal to, or greater than, that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance value per unit time lower than that obtained with the cutoff calibrator is considered negative.

Procedural Limitations
1. A positive result from the assay indicates only the presence of buprenorphine and/or norbuprenorphine, the test is not intended for quantifying the analytes in patient sample.
2. A positive result does not indicate drug abuse.
3. A negative result does not necessarily mean a person did not take amphetamines.
4. Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.
5. The test is not for therapeutic drug monitoring use.
6. There is a possibility that metabolites of other opiate drugs may interfere with the test.
7. The test is designed for use with human urine only.

PERFORMANCE CHARACTERISTICS

The results shown below were obtained with the EasyRA analyzer.

Inaccuracy/Correlation
One hundred and fifty-six (156) clinical urine specimens were tested qualitatively with the Enzymatic Immunoassay (EIA) method on the EasyRA. The correlation results are summarized as follows:
All results were confirmed with LC/MS* and are summarized in the table below:

EasyRA	(<5ng/mL) Negative LC/MS	Near Cutoff (5-10ng/mL) Negative LC/MS	Near Cutoff (10-15ng/mL) Positive LC/MS	(>15ng/ml) Positive LC/MS
Positive(>10ng/mL)	1	0	15	56
Negative(<10ng/mL)	60	20	1	2

% Agreement Negative	**98.80%**
% Agreement Positive	**96.00%**

Imprecision (CLSI, EP5-A2)

Qualitative analysis: Nine samples of Buprenorphine (BUP) spread evenly throughout the range of 0-20.0 ng/mL were prepared in human urine and analyzed in duplicate twice a day for 20 days. The samples were tested in qualitative mode and the absorbance change versus time was also measured for each reading. Typical results (mA/min) are as follows:

Within Run Imprecision (EP5-A2)
Qualitative Results (Cut- off= 10) (n=80)

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	180.0	2.813	1.56%
2.5	190.0	0.805	0.42%
5.0	200.0	0.909	0.45%
7.5	209.6	0.750	0.36%
10.0	217.8	0.768	0.35%
12.5	222.5	0.683	0.31%
15.0	228.1	0.773	0.34%
17.5	234.4	0.709	0.30%
20.0	238.9	0.657	0.27%

Total Imprecision (EP5-A2)
Qualitative Results (Cut- off= 10) (n=80)

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	180.1	3.215	1.79%
2.5	190.1	2.356	1.24%
5.0	200.0	2.827	1.41%
7.5	209.6	2.115	1.01%
10.0	217.8	2.046	0.94%
12.5	222.5	2.344	1.05%
15.0	228.1	2.385	1.05%
17.5	234.4	2.112	0.90%
20.0	238.9	2.008	0.84%

% Agreement of Qualitative Precision Results with Target Values

Samples (ng/mL)	Number Positive	Number Negative	% Agreement
0	0	80	100%
2.5	0	80	100%
5.0	0	80	100%
7.5	0	80	100%
10.0	58	22	N/A
12.5	80	0	100%
15.0	80	0	100%
17.5	80	0	100%
20.0	80	0	100%

Specificity
Various potentially interfering substances were tested for cross-reactivity with the assay on the Hitachii 717. Test compounds were spiked into the drug-free urine calibrator matrix to various concentrations and evaluated against the cutoff calibrator. The table listed the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the concentration of the compounds tested that gave a response below the response of the cutoff calibrator (as negative).

Buprenorphine Compounds:

Compound	Concentration ng/mL	Cross-Reactivity
Buprenorphine	100,000	Positive
Buprenorphine-Glucuronide	100,000	Negative
Norbuprenorphine-Glucuronide	100,000	Negative

Structurally Related Opiate Compounds*:

Compound	Concentration µg/mL	Cross-Reactivity
6-acetylcodeine	100,000	Negative
Codeine	100,000	Negative
Dextromethorphan	100,000	Negative
Dihydrocodeine	100,000	Negative
Heroin	100,000	Negative
Hydrocodone	100,000	Negative
Hydromorphone	100,000	Negative
Levorphanol	100,000	Negative
6-Monoacetylmorphine	100,000	Negative
Morphine	100,000	Negative
M3G	100,000	Negative
M6G	100,000	Negative
Nalorphine	100,000	Negative
Naloxene	100,000	Negative
Norcodeine	100,000	Negative
Noroxycodone HCl	100,000	Negative
Noroxymophine HCl	100,000	Negative
Oxycodone	100,000	Negative
Oxymorphone	100,000	Negative

*There is a possibility that metabolites of the compounds listed above may interfere with buprenorphine immunoassays and cause false results.

Structurally Unrelated Pharmacological Compounds:

Compound	Concentration ng/mL	Cross-Reactivity
alpha-methadol	100,000	Negative
Citalopram	100,000	Negative
EDDP	100,000	Negative
EMDP	100,000	Negative
Fluoxetine	100,000	Negative
Gabapentin	100,000	Negative
Imipramine	100,000	Negative
LAAM	100,000	Negative
Meperidine	100,000	Negative
Methadone	100,000	Negative
Norpropoxyphene	100,000	Negative
Paroxetine	100,000	Negative
Sertraline	100,000	Negative
Tramadol	100,000	Negative

It is possible that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedure errors.

Endogenous Interferents (for reference purposes only)
The following endogenous compounds were spiked into negative urine and the three levels of controls (3, 7, and 13 ng/mL) for the assay. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value 10 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] mg/dL	0 ng/mL ng/mL	3 ng/mL Control ng/mL	7 ng/mL Control ng/mL	13 ng/mL Control ng/mL
Acetone	1000	0.0	3.2	6.5	12.2
Ascorbic Acid	400	0.4	3.3	6.5	13.2
Creatinine	500	0.9	3.8	7.1	13.0
Galactose	10	0.4	3.5	6.4	11.7
γ-Globulin	500	0.0	3.0	8.1	11.2
Glucose	1500	0.0	3.1	7.1	11.5
Hemoglobin	300	0.4	4.0	8.2	13.0
NaCl	6000	0.9	3.9	7.8	13.0
Oxalic Acid	100	0.5	3.3	6.7	11.8

HSA*	500	0.0	3.2	7.1	11.8
Urea	2000	0.0	3.3	7.2	11.6
Ethanol	1000	0.0	3.0	8.1	11.9
pH 3	n/a	0.0	3.3	5.9	13.6
pH 11	n/a	0.0	3.7	8.0	12.7

*Human Serum Albumin

Specific gravity: Urine samples with specific gravity value ranging from 1.001 to 1.027 were tested with the assay in the presence of 0, 3, 7, and 13 ng/mL of norbuprenorphine, and no interference was observed.

Note: All endogenous substances listed above, including specific gravity, were also tested in "qualitative-mode". No interference is observed.

REFERENCES

1 Urine Testing for Drugs of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2 Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, vol. 53, No. 69, ppl 11970 (1988).
3 Baselt, RC: Disposition of toxic drugs and chemicals in man. 5th edition. Chemical Toxicology Institute, Foster City, CA, 2000; pp103-105.
4 Center for Substance Abuse Treatment. Clinical Guidelines for the Use of Buprenorphine in the Treatment of Opioid Addiction. Treatment Improvement Protocol (TIP) Series 40. DHHS Publication No. (SMA) 04-3939. Rockville, Md: Substance Abuse and Mental Health Services Administration, 2004.
5 Cone EJ, Gorodetsky D, Yousefinejad WF, Buchwald WF and Johnson RE (1984) The Metabolism and excretion of buprenorphine in humans. Drug Metab Dispos 12: 577-581.
6 McCance-Katz, et al, "The In Vitro Glucuronidation of Buprenorphine and Norbuprenorphine Determined by Liquid Chromotography-Electrospray Ionization-Tandem Mass Spectrometry". Therapeutic Drug Monitoring, Volume 28, Number 2, April 2006.
7 Rubenstein, K.E., R.S. Schneider, and E.F. Ullman, Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, Biochem Biophys Res Commun, 47, 846 (1972).
8 Yahya, A.M., McElnay, J.C., and D'Arcy, P.F. Drug absorption to glass and plastics, *Drug Metabol Drug Interact*, 6(1):1-45 (1988)
9 McCance-Katz, et. al., The In Vivo Glucuronidation of Buprenorphine and Norbuprenorphine Determined by Liquid ChromotographyElectrospray Ionization-Tandem Mass Spectrometry, *Therapeutic Drug Monitoring,* 28(2):245-251 (2006)

EasyRA Parameters:

	Qualitative
Primary Wavelength	340
Secondary Wavelength	N/A
Reaction Type	Qual. Kinetic
Reaction Direction	Increase
Calibration Curve	Increase
Reagent Blank	N/A
Sample Blank	N/A
Reaction Time	4 Minutes
On-Board Stability	30 Days
Cal Stability	7 Days*

* with anti-evap caps

Manufactured for:

Medica Corporation, 5 Oak Park Drive
Bedford, MA 01730-1413 USA

EC REP Emergo Europe, Prinsessegracht 20
2514 AP The Hague, The Netherlands

008793-901 R3