MEDICA

IVD CE

REF 14249-4 4 x 21 mL/ 9 mL

METHADONE ENZYME IMMUNOASSAY (MTD)
Wedges each contain usable volumes of 21 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE
The EasyRA Methadone (MTD) reagent is intended for the qualitative determination of methadone in human urine at a cutoff value of 300 ng/mL. The assay is designed for prescription use on the EasyRA Clinical Chemistry Analyzer.

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

SUMMARY AND EXPLANATION
Methadone is a synthetic diphenylheptanonylamine opioid that has similar analgesic activity and potency to morphine when administered parenterally. However, unlike morphine, it reliably retains its effectiveness when given orally, and tolerance and physical dependency develop slowly (3, 4). Although methadone is prescribed to relieve chronic pain, its primary application is the detoxification and/or treatment of narcotic or heroin addiction (3–6). The abuse potential of methadone is comparable to that of morphine due to its similar pharmacological activity (3, 5, 7).

Methadone is available in tablets and as a solution for parenteral injection. It is readily absorbed from the gastrointestinal tract when ingested, and metabolized extensively in the liver. Initial N-demethylation results in normethadone, which rapidly undergoes cyclization followed by dehydration to form the 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine, commonly known as EDDP. Further N-demethylation yields a secondary metabolite, the 2-ethyl-5-methyl-3, 3-diphenyl-1-pyrroline (EMDP) (8). The metabolites are secreted in urine or bile along with unchanged drug.

Assay Principle
The EasyRA Methadone (MTD) reagent is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (9). 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, methadone-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody binds to free drug; the unbound methadone-labeled G6PDH then exhibits its maximal enzyme activity.

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

REAGENTS PROVIDED
Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-methadone antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R_2): Contains methadone-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

Precautions and Warnings
- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *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).*
- *Do not use the reagents beyond their expiration dates.*

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 2–8ºC. Opened reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days 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 HANDLING

Urine samples may be collected in plastic or glass containers. Some plastics may adsorb drugs. Use of plastics such as polyethylene is recommended (10). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2–8ºC for up to one week (11). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown methadone analytes in urine are stable at -20ºC up to 384 days (12). Samples should be at room temperature (18–25ºC) 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 forward both samples to the laboratory for testing. *Handle all urine specimens as if they are potentially infectious.*

PROCEDURE

Materials Provided

Medica MTD Reagent Wedge, REF 14249 (Qualitative)

Additional Materials Required

Medica EasyCal Methadone Cutoff Calibrator (Methadone Cutoff, 300 ng/mL), REF 14688
Medica EasyCal Universal Negative Calibrator (Methadone, 0 ng/mL), REF 14799
Medica EasyQC Methadone Negative Control (Methadone, 225 ng/mL), REF 14788
Medica EasyQC Methadone Positive Control (Methadone, 375 ng/mL), REF 14789
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

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Synchron CX®4CEand on the EasyRA Clinical Chemistry Analyzer.

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 300 ng/mL as the cutoff calibrator. Recalibration should be performed if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring of each cutoff level: use the 225 ng/mL and 375 ng/mL controls for the 300 ng/mL cutoff.

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 Methadone Cutoff Calibrator, REF 14688 and Medica EasyCal Universal Negative Calibrator, REF 14799 are required for the calibration of the assay. The calibration interval (30 days 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

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters or contact Medica Corporation technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A positive test result does not always mean a person took illegal drugs and a negative test result does not always mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator which contains 300 ng/mL of methadone is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (ΔmA/min) greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance (ΔmA/min) equal to or 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 methadone. The test is not intended for quantifying this single analyte in samples.
2. A positive result does not necessarily indicate drug abuse.
3. A negative result does not necessarily mean a person did not take illegal drugs.
4. Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogeneous or exogeneous interferents) may influence the urine test result.
5. Positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.
6. The test is designed for use with human urine only.
7. The test is not for therapeutic drug monitoring.

Typical Performance Characteristics

The results shown below were obtained with the Synchron CX®4CE clinical chemistry analyzer and validated on an EasyRA Clinical Chemistry Analyzer.

Precision:

Qualitative analysis: The three calibrators and two levels of controls were evaluated. Typical results (ΔmA/min) are as follows:

(mA/min)	Within Run (n=21)			Run-to-Run* (n=12)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	209.4	1.0	0.5 %	209.5	1.1	0.5 %
225 ng/mL	272.8	1.1	0.4 %	271.4	2.0	0.7 %
300 ng/mL	293.4	0.7	0.3 %	292.3	1.9	0.7 %
375 ng/mL	308.2	0.9	0.3 %	307.2	2.6	0.8 %
1000 ng/mL	344.9	1.1	0.3 %	344.1	2.0	0.6 %

**Run-to-Run testing completed over 3 weeks*

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be
15 ng/mL.

Accuracy: One hundred and sixty (160) clinical urine specimens were tested with current EIA, 49 samples were positive, and 111 samples were negative. All positive samples were confirmed with GC/MS results.

Cutoff Value (300 ng/mL)	GC/MS	MTD EIA	% Agreement with Predicate
# Positive Samples	49	49	100 %
# Negative Samples	111	111	100 %
Total # of Samples	160	160	N/A

In addition to the above study, 19 diluted clinical samples with a methadone concentration ranging from 205 ng/mL to 434 ng/mL (determined by GC/MS) were evaluated with the EIA assay. The eight samples with methadone GC/MS values greater than the cutoff (ranging from 305 to 434 ng/mL) tested positive by EIA. Among the 11 samples with methadone GC/MS below the cutoff (ranging from 205 to 288 ng/mL), three samples were found negative while eight samples tested positive by the EIA. The GC/MS concentrations of methadone in these eight samples range from 214 ng/mL to 288 ng/mL. Each of these samples also contained a substantial amount of the primary methadone metabolite EDDP (ranging from 247 ng/mL to 825 ng/mL).

Analytical Recovery: Analytical recovery was evaluated by spiking known concentrations of methadone to negative urine samples. In qualitative analysis, the assay correctly identified spiked samples containing more than 300 ng/mL of methadone (n=25, spiked levels equal to or higher than Level 2 Control, 375 ng/mL) as positive, and those containing less than 300 ng/mL of methadone (n=25, spiked levels equal to or less than Level 1 Control, 225 ng/mL) as negative.

Specificity

Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix to various concentrations and evaluated against the cutoff calibrator.

The table below lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative).

Structurally Related Methadone Compounds:

Compound	Concentration (µg/mL)	Cross-Reactivity
Methadone	0.30	Positive
LAAM.HCl	10	Positive
(-) α-Methadon.HCl	8	Positive
Nor-LAAM.HCl	10	Negative
EDDP.HI	100	Negative
EMDP.HCl	100	Negative

Structurally Unrelated Pharmacological Compounds:

Compound	Concentration (µg/mL)	Cross-Reactivity
Acetaminophen	1000	Negative
Acetylsalicylic acid	1000	Negative
Amitriptyline	1000	Negative
Amobarbital	1000	Negative
Amphetamine	1000	Negative
Benzoylecgonine	1000	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Cocaine	1000	Negative
Codeine	1000	Negative
Dextromethorphan	1000	Negative
Ecgonine	1000	Negative
Ephedrine	1000	Negative
Ibuprofen	2000	Negative
Imipramine	1000	Negative
Lidocaine	1000	Negative
Meperidine	1000	Negative
Methamphetamine	1000	Negative
Methaqualone	1000	Negative
Morphine	1000	Negative
Nortriptyline	1000	Negative
Oxazepam	1000	Negative
Phencyclidine	1000	Negative
Phenobarbital	1000	Negative
Promethazine	1000	Negative
Propoxyphene	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Valproic Acid	1000	Negative

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

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13. Nichols, J. Instrument Validation: The Road to Success. CLN's Lab 2004: From Basic to Advanced Series. 14–16 (2004).
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EasyRA Clinical Chemistry Analyzer

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	2.1 Minutes
On-Board Stability	30 Days
Cal Stability	30 Days

with anti-evaporation caps

Performance Characteristics
The results shown below were obtained with the EasyRA analyzer.

Inaccuracy/Correlation
Ninety-nine (99) clinical urine specimens were tested qualitatively with the Methadone Enzymatic Immunoassay (EIA) method on the EasyRA. All results were confirmed with an LC/MS method and are summarized in the table below:

	(0–300ng/mL) Negative	**(>300ng/mL) Positive**
EasyRA	**LC/MS**	**LC/MS**
Negative (<300ng/mL)	55	1
Positive (>300ng/mL)	3	40

% Agreement Negative	97.6%
% Agreement Positive	94.6%

Imprecision (CLSI, EP5-A2)
Qualitative analysis: Precision of methadone, samples (Negative, 225 ng/mL and Positive, 375 ng/mL) was performed qualitatively (mAbs/min) on one Medica EasyRA Analyzer. Each of the precision samples was analyzed 20 times per run. The mean, standard deviation and the %CV were calculated for within run precision.

Within-Run Imprecision (EP5-A2)
Qualitative Results (Cutoff 300 ng/mL)
Qualitative Results (n=20)

Samples (ng/mL)	**Mean (mAbs/Min)**	**SD (mAbs/Min)**	**%CV**
225	182.6	0.5	0.3%
375	202.6	0.9	0.4%

% Agreement of Qualitative Precision Results with Target Values

Sample	**Acceptance Criteria**	**Results**
225 ng/mL	100%	Negative
375 ng/mL	100%	Positive

Qualitative analysis: Two samples of methadone were prepared in human urine and analyzed 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. The study followed the protocol defined in EP5-A2. Typical results are as follows:

Total Imprecision (EP5-A2)
Qualitative Results (Cutoff 300 ng/mL)
Qualitative Results (n=40)

Mean (ng/mL)	SD	%CV
216.6	13.4	6.2%
356.6	11.4	3.2%

% Agreement of Qualitative Precision Results with Target Values

Sample	Acceptance Criteria	Results
216.6 ng/mL	100%	Negative
356.6 ng/mL	100%	Positive

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