Rapid EDDP Test (RAP-4712)

Revised 3 Apr. 2007 **USA: RUO**

For the Qualitative Assessment of EDDP in Human Urine

INTENDED USE

The Rapid EDDP test is an immunochromatographic one-step test intended for the qualitative determination of methadone's primary metabolite, 2-Ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in human urine specimen.

Rapid EDDP Test may be used as a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography / Mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the Substance Abuse Mental Health Services Administration (SAMHSA). Clinical consideration and professional judgment should be applied to any test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION

2-Ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) is the primary metabolite of methadone. Methadone is a controlled substance and is used for detoxification and maintenance of opiate dependant patients. Patients on methadone maintenance may exhibit methadone (parent) levels that account for 5-50% of the dosage and 3-25% of EDDP in urinary excretion during the first 24 hours. The detection of EDDP is more beneficial than traditional methadone screening, in that EDDP exists only in urine from individuals that ingested methadone. The tampering of specimens by spiking the urine with methadone can be prevented. Secondly, renal clearance of EDDP is not affected by urinary pH, therefore the EDDP test provides a more accurate result of methadone ingestion than the methadone parent screen.

TEST PRINCIPLE

RaspidEDDP Test is based on the principle of the highly specific immunochemical reactions of antibodies and antigens, which are used for the analysis of specific compounds in human urine. The drug-protein conjugate competes for limited antibody binding sites with drugs that may be present in the urine. When drug is present in the urine specimen, it competes with drug protein conjugate for the limited amount of antibody-dye conjugate. If the amount of free drug is equal to or greater than the cut-off, it will prevent binding of the drug protein conjugate to the antibody-dye conjugate. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result.

A control line is present in the test window to serve as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed properly.

REAGENTS AND MATERIALS PROVIDED

The test consists of an individually sealed test cassette with a desiccant. The amount of each coated antigen and/or antibody on the strip is less than 1.0 mg for antigen conjugate and is less than 1.0 mg for goat anti-mouse IgG antibody for each specific drug. The specific Test zone contains drug-bovine protein conjugates, while the Control zone contains Goat anti-mouse IgG antibody. The Conjugate pad contains mouse monoclonal anti-drug antibody. Instruction for Use is also provided.

MATERIALS REQUIRED BUT NOT PROVIDED

- Urine collection container.
- Timer or clock.

WARNINGS AND PRECAUTIONS

- For diagnostic use only.
- Do not use beyond the expiration date indicated on the product.
- Use separate clean tips for different specimens. Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling specimens and thoroughly wash hands after handling.
- All patient samples should be handled as if they are capable of transmitting diseases. Observe established procedures for proper disposal of specimens and used test devices.
- The test is a humidity sensitive device and should remain in its sealed pouch until ready for use.
- Use a new urine specimen cup for each sample to avoid cross-contamination.

STORAGE AND STABILITY

- The test should be stored at 2-30^{O}C and is effective until the expiration date stated on the package.
- The product is humidity-sensitive and should be used immediately after being open.
- Any improperly sealed product should be discarded.

SPECIMEN COLLECTION AND PREPARATION

- Fresh urine sample does not require any special handling or pretreatment.
- Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, the urine specimen may be stored at 2-8 °C or frozen up to 7 days. Specimens should be brought to room temperature before testing.
- Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing.
- Avoid contact with skin by wearing gloves and proper laboratory attire.

QUALITY CONTROL

- Good Laboratory practice recommends the daily use of control materials to validate the reliability of the test device. Control materials should be assayed as clinical specimens to challenge the assay cutoff concentration, e.g., 25% above and below the cutoff concentrations.
- If control values do not fall within establish range, assay results are invalid.
- Control materials, which are not provided with this test kit, are commercially available.
- The test provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded. The test should be repeated. The presence of a control band in the control region serves as verification that (1) sufficient volume has been added and (2) proper flow has occurred.

TEST PROCEDURE:

For test strip

1. Bring all materials and specimens to room temperature.
2. Remove the test strip from the sealed foil pouch.

3. Dip the strip into the urine specimen with the arrow pointing toward the sample. The sample level should not be higher than the arrow pointed maximum line.
4. Hold the strip in the urine until a reddish color appears at the test area (approximately 20 seconds).
5. Withdraw the strip and place it face up on a clean, non-absorptive surface or leave the strip in urine if the urine level is not higher than arrow pointed maximum line.
6. Read the results at 5 minutes after adding the sample.
   Do not interpret the result after 5 minutes.

For test card

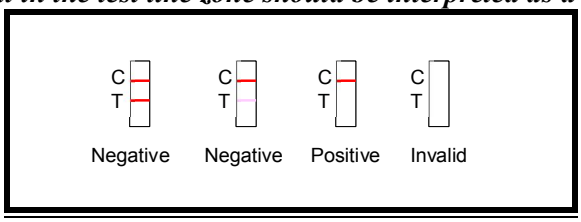
1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch.
3. Place the transfer pipette in the specimen and depress the bulb to withdraw a sample.
4. Hold the pipette in a vertical position over the sample well of the test card and deliver 2-3 drops (100-150 µl) of sample into the sample well.
5. Read the result at 5 minutes after adding the sample.
   Do not interpret the result after 5 minutes.

INTERPRETATION OF TEST RESULTS

- **Negative: Two colored bands form on any strip of the card.**
  The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative result for that particular test(s). The negative result does not indicate the absence of drug in the specimen; it only indicates the level of tested drug in the specimen is less than the cut-off level**.**

- **Positive: One colored band forms on any strip of the card.**
  One colored band appears in control line zone. No colored band is found in test line zone. This is an indication the level of tested drug(s) in the specimen is above the cut-off level.

- **Invalid: NO colored band forms.**
  If there are no colored bands in the control line zone of any strip, the test result is invalid. *Retest the sample with a new device.*
  - ***Note: A borderline* (±) *result in the test line zone should be interpreted as a negative result.***



LIMITATIONS OF THE PROCEDURE

- The assay is designed for use with human urine only.

- A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication.
- There is a possibility that technical or procedural error as well other substances in certain foods and medicines may interfere with the test and cause false results.
- Please refer to "SPECIFICITY" section for lists of substances that will produce either positive results, or that do not interfere with the test performance.
- If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED VALUES

- The test is a qualitative assay, which only identifies the drug(s) concentration in human urine is higher or lower than the cut-off concentration. The exact concentration of the drug(s) cannot be determined by this assay.
- The test is intended to distinguish a negative result from a presumptive positive result.
- All positive results must be confirmed by using an alternate method, preferably GC/MS.

TEST PERFORMANCE

- **Accuracy**

  The accuracy of the methadone metabolite (EDDP) test was evaluated in comparison to GC/MS method at a cut-off of 100 ng/mL EDDP. One hundred and sixty (160) specimens with EDDP concentration confirmed by GC/MS were evaluated.

	(-)		(+)		
Rapid EDDP Test	**Negative by GC/MS**	**Near Cut-off NEG** (between –25% and C/O)	**Near Cut-off POS** (between C/O and +25%)	**GC/MS POS** (greater than +25% C/O)	**Percent agreement with GC/MS**
Positive	0	2	8	70	98%
Negative	70	8	2	0	98%
Total	70	10	10	70	n=160

Positive agreement = 98% Negative agreement = 98%

- **Sensitivity**

  The cut-off concentrations (sensitivity level) of the test are determined to be 100 ng/mL of EDDP.

- **Precision**

  The precision of the test was determined by conducting the test with spiked controls and interpreted the results to verify the random error of visual interpretation. Acceptable result criteria are stated as "+/-" for this level (cut-off). The results of the precision study are summarized below.

DRUG	CONTROL (ng/mL)	#NO	# +	# +/-	# -
EDDP	50 ng/mL	56	0		56
	75 ng/mL	160	14		146

(100 ng/mL)	125 ng/L	106	106		0
	150 ng/L	56	56		0

- **Specificity**
  The specificity of the test was evaluated by adding various drugs, drug metabolites, and other compounds that were likely to be present in the urine. All compounds were prepared in drug-free human urine.

1. **Interference with pH, specific gravity and endogeneous substances:**
   The test performance at cut-off level is not affected when pH and specific gravity ranges are at 4.5 to 9.0 and 1.005 to 1.035 respectively. The following endogeneous substances were also tested and results confirmed that there was no interference with the panel tests at the listed concentrations.

Glucose	2000 mg/dL	Human albumin	2000 mg/dL
Human hemoglobin	10 mg/dL	Urea	4000 mg/dL
Uric acid	10 mg/dL		

2. **Cross-reactivity with structurally similar compounds:**
   The following table lists compounds that are detected by the DOA panel test, which produce *positive* results when tested at levels equal or greater than the concentrations listed below:

Compounds	**Cut-off**	**Cross reactivity**
EDDP	100 ng/Ml	100%
EMDP	200,000 ng/mL	0.05%
Methadone	500,000 ng/mL	0.02%

3. **Cross-reactivity with other potential interfering substances:**
The following compounds show no cross-reactivity at concentrations up to 100 µg/mL unless specified.

Acetaminophen	Acetylsalicylic acid	Amikacin	Amitriptyline
Amobarbital	Amphetamine	Arterenol	Aspartame
Ascorbic acid	Atrophine	Benzoic acid	Benzoylecgonine
Butabartital	Caffeine	Camphor	Chlopheniramine
Chloroquine	Cocaine	Cortisone	Deoxyephedrine
Dextromethorphan	Diazepam	Digitoxin	Digoxin
Diphenhydramine	Ecgonine	Ecgonine methyl ester	Ephedrine
Epinephrine	Gentisic acid	Guaiacol glycer ester	Histamine
Homatrophine	Hydrochlorothiazide	Ibuprofen	Imipramine
Isoproterenol	Ketamine	Lidocaine	3,4±MDA
Methamphetamine	eperidine	Methaqualone	Methylphenidate
Morphine	Neomycin	Niacinamide	Oxazepam
Perphenazine	Penicillin G	Phencyclidine	Phenobartital
Phenylethylamine-α	Phenylpropanolamine	Promethazine	Pseudoephedrin
Quinine antidine	Salicylic acid	Secobarbital	Tetracycline

Tetrahydrozoline Theophyline 11-nor-Δ^8-THC-9-COOH (10 µg/ml)
11-nor-Δ^9-THC-9-COOH (10 µg/ml) Thioridazine Trifluoperazine
Tyramine Tryptophan

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