OneStep EDDP RapiCard™ InstaTest

REF 121005-1-21

IVD See external Label 2°C 30°C 1 Test

Sensitivity	**100 ng/ml**
Specificity	**100 %**

INTENDED USE

The Cortez Diagnostics, Inc. OneStep EDDP RapiCard™ InstaTest 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. The determine limit (cut-off) is 100 ng/ml.

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.

TEST PRINCIPLE

EDDP 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 cutoff, 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.

SPECIMEN COLLECTION AND PREPARATION

It is required that approximately 150 µl of sample for each test. Fresh urine specimens do not need any special handling or treatment. Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2-8oC or frozen up to 7 days. Specimens should be thawed and 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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Instructions for use.
2. EDDP test device. 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.
3. Test zone: contains EDDP bovine protein antigen conjugates.
4. Control zone: contains Goat anti-mouse IgG antibody.
5. Conjugate pad: contains mice monoclonal anti-EDDP antibody.

Materials required but not provided

1. Urine collection container.
2. Timer or clock.

ASSAY PROCEDURE

1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch and put it on the horizontal surface.
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 card and deliver 3 drops (120-150ul) of sample into the sample well.
5. Read the results at 5 minutes after adding the sample. ***Do not interpret the result after 10 minutes.***

RESULTS

- **Negative:** Two colored bands form. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative results. The negative result indicates that the EDDP concentration in the specimen is either zero or less than cut-off level.
- **Positive:** One colored band forms. One colored band appears in control line zone. No colored band is found in test line zone. This is an indication that the EDDP level in the specimen is above the cut-off level.
- **Invalid:** If there is no colored band in control line zone, the test result is invalid. Retest the sample with a new device.

Note: *A borderline (+/-) in test line zone should be considered negative result.*

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QUALITY CONTROL

Good Laboratory Practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. 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 Rapid Drugs of Abuse 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 and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

PERFORMANCE CHARACTERISTICS

A. 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.

	(-)		(+)		
Cortez EDDP Test	Negative By GC/MS	Near Cut-off NEG (between -25% and C/O)	Near Cut-off N POS (between -25% and C/O)	GC/MS POS (greater than + 25% C/O)	Percent agreement with GC/MS
Positive	0	2	8	70	97.5 %
Negative	70	8	2	0	97.5 %
Total	70	10	10	70	**N =160**

Positive % agreement: 97.5, Negative % agreement: 97.5.
Four (4) specimens were found discrepant between the EDDP and GC/MS method. When compared those data, 100% (4 out of 4) of the discrepancy specimens were found between -25% cut-off and +25% cut-off concentration (75 – 125 ng/ml).

B. Sensitivity

The cut-off concentration (sensitivity level) EDDP test is determined to be 100 ng/mL of EDDP.

C. Precision

The precision study was performed by three individuals observing the test result to determine the random error of visual interpretation. The test results were found to have no significant differences between the three observers.

Device	Control Con. ng/ml	No. of Tested	No.of negative			No.of borderline *			No.of positive		
			1*	2*	3*	1*	2*	3*	1*	2*	3*
	50	120	40	40	40						
	75	120	31	28	30	9	12	10			
EDDP	100	120				11	14	10	29	26	30
	125	120				1	1	2	39	39	38
	150	120							40	40	40

The precision study was performed by three individuals observing the test results to determine the random error of visual interpretation. The results were found to have no significant differences between three observers.

D. Specificity

The specificity for EDDP test was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine.

1. Interference Testing

The EDDP test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.5 to 9.0 and 1.005 to 1.035.

The following substances were tested and confirmed not to interfere with RapidEDDP test 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. Specificity

The following table lists compounds that are detected by Cortez BAR test which produced positive results when tested at levels equal or greater than the concentrations listed below:

Compounds	Cut-off (ng/ml)	Cross reactivity (%)
EDDP	100 ng/m/	100 %
EMDP	200,000 ng/ml	0.05 %
Methadone	500,000 ng/ml	0.02%

Each listed substance that commonly found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml.

Acetaminophen
Amitriptyline
Aspartame
Camphor
Deoxyephedrine
Diphenhydramine
Epinephrine
Hydrochlorothiazide
Isoproterenol
Methamphetamione
Neomycin
Penicillin G
Promethazine
Tetrahydrozoline
11- nor-Δ^8–THC-9-COOH (10 µg/ml)
Tryptophan

4-Acetamidophenol
Amobarbital
Ascorbic acid
Chloroquine
Dextromethorphan
Ecgonine
Gentisic
Homatrophine
Ketamine
3,4 ±MDMA
Niacinamide
Phencyclidine
Pseudoephedrine
Quinine antidine
Theophyline
Tyramine

Acetylsalicylic Acid
Amphetamine
Atrophine
Chlopheniramine
Digitoxin
Ecgonine methyl ester
Guaiacol glycer ester
Imipramine
Lidocaine
Methaqualone
Oxazepam
Phenylethylamine-α
Salicylic acid
11- nor-Δ^8–THC-9-COOH (10 µg/ml)
Thioridazine

Amikacin
Alterenol
Caffeine
Cortisone
Digoxin
Ephedrine
Histamine
Ibuprofen
Meperidine
Methylphenidate
Perphenazine
Phenylpropanolamine
Tetracycline
Trifluoperazine

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LIMITATION OF 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 "SPECIFICITY" section for lists of substances that will produce either positive results, or that do not interfere with 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 Cortez Diagnostics, Inc. OneStep Rapid EDDP test is a qualitative assay, which only identifies the EDDP concentration in human urine is higher or lower than the cut-off concentration. The exact concentration of the EDDP 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.

PRECAUTION

1. For in vitro diagnostic and forensic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. Use a new urine specimen cup for each sample to avoid cross contamination.

STORAGE

The test device should be stored at 2 to 30^{O}C and will be 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.

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