



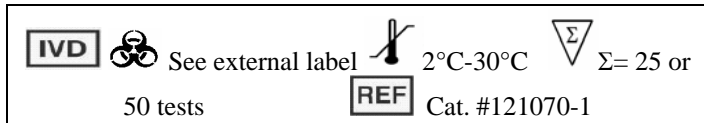
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ONESTEP METHADONE URINE RAPICARD INSTATEST

INTENDED USE

The Cortez Diagnostics, Inc. Rapicard OneStep Methadone Test is a rapid, competitive binding immunoassay for the qualitative determination of methadone in urine at or above the cut-off level of 300 ng/ml. OneStep Methadone Test is not intended to monitor drug levels, but only to screen for the presence of methadone and its major metabolites.

Note: The test provides only preliminary data, which should be confirmed by other methods, such as gas chromatography/mass spectrometry (GC/MS). Clinical considerations and professional judgement should be applied to any drugs of abuse result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION OF THE TEST

The OneStep Methadone test is an easy, fast and visually read competitive binding immunoassay method for screening without the need for instrumentation to arrive at determination. The test system employs unique polyclonal antibodies to selectively identify methadone in urine samples with a high degree of sensitivity.

Methadone is a commonly used substitute for heroin or other abused opiates in drug maintenance treatment clinics.²⁻⁶ It is administered orally or intravenously and is metabolized in the liver. Excretion occurs through the kidneys in concentrations of 50 mg/dL or greater. Twenty-four hours after a dose is administered, urine levels of methadone maintenance patients typically range from 1 to 5 µg/ml.⁷⁻⁸

Historically, a number of techniques have been employed for methadone detection in biological samples, including gas chromatography/mass spectrometry (GC/MS), ultraviolet spectroscopy, thin-layer chromatography, enzyme immunoassay and radioimmunoassay.

The mandated allowable level for Methadone is set at 300 ng/ml in urine by the confirmatory GC/MS method specified by the National Institute on Drug Abuse (NIDA).

PRINCIPLE OF THE TEST

The OneStep Methadone Test consists of a chromatographic absorbent device in which the drug or drug metabolites in the sample compete with a drug conjugate immobilized on a porous membrane for limited antibody sites. As the test sample flows up through the absorbent device, the free drug in

the specimen competes with immobilized antigen conjugate in the test zone by binding to the antibody-dye conjugate forming an antibody-antigen complex and preventing the formation of a rose-pink color band when the drug is at or above the detection level of 300 ng/ml.

In the case where free drug in the sample is below the detection level of 300 ng/ml, antibody-dye conjugate is free to bind to the immobilized antigen in the test zone, producing a rose-pink color band. Furthermore, unbound dye conjugate binds to the reagent in the control zone, producing a rose-pink color band in said zone, demonstrating that the reagents and device are functioning correctly.

A **NEGATIVE** specimen produces two distinct color bands, one in the test zone and one in the control zone. A **POSITIVE** specimen produces only one color band in the control zone and none in the test zone.

REAGENTS AND MATERIALS PROVIDED

1. Test Cassette. The test device contains membrane-immobilized reagents in a protein matrix containing sodium azide.
2. Droppers. A transfer pipette is sealed inside each foil pouch with the test device.
3. Urine Cups (optional)
4. Positive Control (optional)
5. Negative Control (optional)
6. Test Instructions

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer.
2. Sample collection containers.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic and *professional* use only.
2. Do not use the test cassette beyond the expiration date.
3. Urine specimens may be infectious; properly handle and dispose of all used reaction devices in a biohazard container.
4. Visually inspect the foil package to insure it is intact. If the package is not intact, discard the device.

STORAGE AND STABILITY

Store test kit below 28°C; do not freeze. Refer to the expiration date for stability.

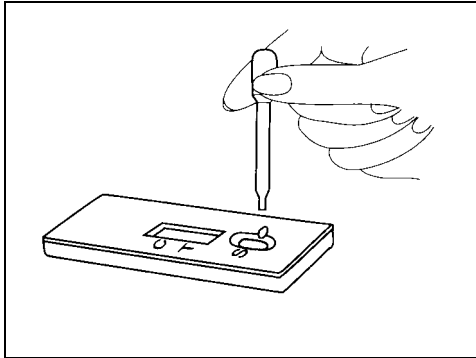
SAMPLE COLLECTION AND PREPARATION

Samples must be collected in a clean, dry container, either plastic or glass, without any preservatives. Urine specimens may be refrigerated (2°-8°C) and stored up to forty-eight hours, or frozen (-20°C or below) prior to assaying. If samples are refrigerated or frozen, they should be allowed to come to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged or allowed to settle, so that clear aliquots can be obtained for testing.

TEST PROCEDURE

1. Bring the test components and urine sample to room temperature (15°-28°C). Do not open the foil pouch until ready to begin testing.

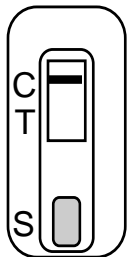
- Open the foil pouch at the notch and remove the test device. Place the device on a clean, flat surface.
- Holding the dropper vertically, add four drops of urine (~120µl) to the sample well "S."



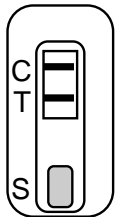
- Wait 5 minutes and read the result.

IMPORTANT: Do not interpret a test result after more than five minutes. Waiting longer than five minutes may cause inaccurate interpretation. To avoid confusion, discard the test device after reading the result at five minutes.

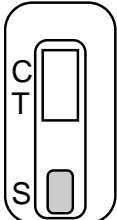
INTERPRETATION OF RESULTS



1. **Positive.** One rose pink color band appears in the Control Zone ("C"), with no apparent band in the Test Zone ("T"). A positive result indicates that the methadone level is at or above 300 ng/ml.



2. **Negative.** Two rose pink color bands appear--one in the Control Zone ("C") and one in the Test Zone ("T"). A negative result indicates that the methadone level is below 300 ng/ml.



3. **Invalid.** No rose pink color bands appear, or a band appears in the Test Zone ("T") but not in the Control Zone ("C"). An invalid result may be due to improper testing procedures or deterioration of the kit components. Repeat the assay sequence using a new device.

Note: There is no meaning attributed to line color intensity or width.

QUALITY CONTROL

An internal procedure control has been incorporated into the test to ensure proper kit performance and reliability.

The use of an external control is recommended to verify proper kit performance. Quality control samples should be tested according to quality control requirements established by the testing laboratory.

LIMITATIONS OF THE TEST

- This product is designed for use with human urine only.
- Although the test is very accurate in detecting Methadone, there is a possibility that false results will occur due to the presence of interfering substances in the urine and/or factors beyond the control of the manufacturer, e.g. technical or procedures errors associated with the testing.
- The test is a qualitative screening assay and is not for determining quantitative concentration levels or the level of intoxication.
- Adulterants such as bleach or other strong oxidizing agents, when added to urine specimens, may produce erroneous test results regardless of the analysis method used. If adulteration is suspected, obtain another urine specimen and retest.

PERFORMANCE CHARACTERISTICS

- Sensitivity.** The OneStep Methadone Test detects methadone and the major metabolites of methadone in urine at concentrations equal to or great than 300 ng/ml which is suggested by NIDA for the immunoassay method.
- Specificity.** A study was conducted with the OneStep Methadone Test to determine the cross-reactivity of non-related compounds with the test at concentrations much higher than normally found in the urine of people using or abusing them. No cross-reactivity was detected with the substances listed in **Table-I**.

A separate study was conducted to determine the cross-reactivity of methadone-related compounds with the test. Substances listed in **Table-II** produced results approximately equivalent to the cut-off level for methadone.

Table- I: Compounds that produce negative results at indicated concentrations (µg/ml):

4-Acetamidophenol	100	Estrone-3-sulfate	100	Nylidrin	190
Acetophenetidin	100	Ethyl-p-Aminobenzoate	180	d,l-Octopamine	190
N-Acetylprocainamide	200	Flunitrazepam	100	Oxalic acid	400
Acetylsalicylic acid	300	Flurazepam	100	Oxazepam	100
Alphenol	100	Furosemide	150	Oxolinic Acid	110
Alprazolam	100	Genistic acid	120	Oxycodone	100
Amirapryline	100	Guaiacol Glyceryl		Oxymetazoline	100
Amobarbital	100	Ester Carbonate	220	Papaverine	120
Amoxicillin	130	Glucuronic acid	200	Penicillin-G	120
l-Amphetamine	100	5-Hydroxytryptamine	100	Pentabital	100
d,l-Amphetamine	100	Hyppuric acid	200	Penopropfen	200
Ampicillin	200	Hydralazine	200	Perphenazine	140
Apomorphine	100	Hydrochlorothiazide	100	Pheneldimetrazine	100
Aprobarbital	100	Hydrocodone	100	Phenelzine	350
ASP-PHE Methyl Ester	100	Hydrocortisone	130	Phenobarbital	100
Atropine	100	O-Hydromorphone	100	Phentermine	100
Barbital	100	Hydroxyhippuric acid	140	l-Phenylephrine	100
Benzilic Acid	300	p-Hydroxymethamphetamine	100	(±)-Phenylpropanolamine	100
Benzoic Acid	280	3-Hydroxystyramine	160	β-Phenylethylamine	180
Benzoylcegonine	100	Ibuprofen	100	Prazepam	100
Benzphetamine	100	Imipronine	190	Prednisolone	150
Bromazepam	100	(-) Isoproterenol	120	Prednisone	120
Butabarbital	100	l-Prmazidol	120	Progylne	100
Butalbital	100	Isoxsuprine	130	Promazine	120
Cannabidiol	100	Ketamine	130	Propiomazine	220
Clonazepam	100	Ketoprofen	140	d-Propoxyphene	100
Chloral Hydrate	100	Labetalol	100	d-Pseudoephedrine	100
Chlorazepate	750	Lidocaine	100	Quinidine	100
Chloridiazepoxide	100	Loperamide	150	Quinine	100
Chlorothiazide	320	Lorazepam	100	Ranitidine	200
Chlorpromazine	100	Lormetazepam	100	Salicylic acid	100
Chloroquine	330	Maprotiline	140	Secobarbital	100
Cholesterol	160	Medazepam	100	Sulfamethazine	150
Clobazem	100	Meperidine	100	Sulindac	120
Clomipramine	230	Meprobamate	100	Tenazepam	100
Clonazepam	100	Methaqualone	100	Tetracycline	200
Clonidine	100	(S) 6-Methoxy-α-Methyl-2-	250	ΔTetrahydrocannabinol	100
Cocaine	100	Naphthaleneacetic acid	100	ΔTetrahydrocannabinol	100
Codaine	100	Methylphenidate	100	Tetrahydrocortisone	100
Cortisone	120	Methpyrrolon	100	Thebaine	100
(-) Cotinine	100	Morphine-3-β-D-glucuronide	100	Thiamine	120
Creatinine	190	(±) 3,4-Methylenedioxy	100	(±) Thiopental	100
Desmethyldiazepam	100	Methamphetamine	100	Thioridazine	110
Deoxycorticosterone	170	(±) 3,4-Methylenedioxy	100	d,l-Thyroxine	120
Diazepam	100	Amphetamine	100	Tolbutamide	100
Diclofenac	100	Methoxyphenamine	150	Triamterene	120
Diflunisal	100	Nalidixic acid	130	Triazolam	100
Digoxin	150	Nalorphine	100	Trifluoperazine	220
4-(Dimethylamino)antipyrine	100	Naloxone	100	Trimethoprim	180
DL Glutethinide	100	Naltrexone	100	Trimipramine	190
Doxepin	100	Niacinamide	170	Tryptamine	150
(+) Ephedrine	130	Nifedipine	140	d,l-Tryptophan	170
(±) Ephedrine	160	Nitrazepam	100	Tyramine	120
(-) γ-Ephedrine	290	Norcodeine	100	d,l-Tyrosine	250
(-) Ephedrine	130	Norethindrone	100	Uric acid	230
Erythromycin	150	d-Norpropoxyphene	100	Verapamil	150
b-Estradiol	110	Noscapine	100	Zomepirac	130

4. **Precision.** The precision was determined by replicate assays of three different patient urine samples with kits from three different production lots. The resultant data indicated 100% precision for the duplicates within each lot and no appreciable interlot variation when testing both positive and negative spiked samples across three (3) different lots of devices.

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Table-II: Concentration of methodadone-related compound producing a positive response approximately equivalent to the methadone cut-off set for the test.

Compound	Concentration (ng/ml)
Methadone	300
Doxylamine	10,000
Laam	10,000
n-methyl-diethanolamine	50,000
Pentazocaine	50,000
Tetrahydrozoline	50,000
Dextromethorphan	100,000
Diphenhydramine	100,000
Leuorphanol	100,000
Promethizine	100,000

3. **Accuracy.** An independent correlation study was performed using positive and negative urine specimens. Each urine specimen was tested with the OneStep Methadone Test and a commercially available test (Syva EMIT II). Positive results were confirmed by GC/MS. The results are summarized as follows:

	<u>Syva EMIT II Positive</u>	<u>Syva EMIT II Negative</u>
OneStepRapidcard Positive	32	0
OneStepRapidcard Negative	0	78

When compared to EMIT II the relative sensitivity was 100%. The relative specificity was 100%. The concordance of the combined data with respect to the EMIT II was 100%.