Revised 17 July 2008 **IVD**

FOR THE QUALITATIVE ASSESSMENT OF METHADONE IN HUMAN URINE

For *in vitro* Diagnostic and Forensic Use

INTENDED USE

The DRG® Rapid MTD test is an immunochromatography based one step in vitro test. It is designed for qualitative determination of methadone in human urine specimens above a cut-off level of 300 ng/ml. The assay may be used in the point of care setting.
This assay provides only a preliminary analytical test result. A more specific alternative chemical method, such as GC/MS, must be used in order to obtain a confirmed analytical result. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION

Methadone is a synthetic opioid, clinically available. It is used clinically for the treatment of severe pain and in maintenance programs for morphine and heroine addicts.
Methadone acts on the central nervous and cardiovascular systems to produce respiratory and circulatory depression. Methadone also produces miosis and increases the tone of smooth muscle in the lower gastrointestinal tract while decreasing the amplitude of contractions. Acute higher doses induce analgesia, sedation, respiratory depression and coma.
After methadone administration, the major urinary excretion products are methadone and its metabolites, EDDP and EMDP. Large individual variations in the urine excretion of methadone are output of methadone from 5-22%. Typically, following a 5 mg oral dose, methadone and EDDP account for 5% of the dose in the 24-hour urine. In those individuals on maintenance therapy, methadone may account for 5 to 50% of the dose in the 24-hour urine and EDDP may account for 3 to 25% of the dose.

TEST PRINCIPLE

The DRG® Rapid MTD test is based on the principle of specific immunochemical reaction between antibodies and antigens to analyze particular compounds in human urine specimen. The assay relies on the competition for binding mouse anti-methadone monoclonal antibody between methadone-protein conjugate and free drug which may be present in the urine specimen being tested.
When methadone is present in the urine specimen, it competes with methadone-protein conjugate for the limited amount of monoclonal antibody-colloidal gold conjugate. When the amount of methadone is equal or more than the cut-off, it will prevent the binding of methadone-protein conjugate to the antibody. 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 composed of Goat anti-Mouse IgG antibody is present in the test window to work 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 appropriately.

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MATERIALS PROVIDED

- Instructions for use.
- DRG® Rapid MTD 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.

Test zone: contains Methadone bovine protein antigen conjugates.
Control zone: contains Goat anti-mouse IgG antibody.
Conjugate pad: contains about 2 µg of colloidal gold-mouse anti-methadone monoclonal antibody conjugate.

MATERIALS REQUIRED BUT NOT PROVIDED

- Urine collection container.
- Timer or clock.

STORAGE AND STABILITY

The test device should be stored at 2 to 30ºC 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.

PRECAUTIONS

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

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.

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

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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.

The control line does not appear, the test should be discarded and the obtained result is invalid.
You should always follow local, state and federal guidelines for running QC.

PROCEDURE

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.
7. Do not interpret the result after 5 minutes.

INTERPRETATION OF 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 methadone 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 methadone 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.

LIMITATION OF PROCEDURE

The assay is designed for use with human urine only. This test has not been evaluated in a point of care setting. 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 RESULTS

The DRG® Rapid MTD Test is a qualitative assay. It identifies methadone in human urine at a concentration of 300 ng/ml or higher. The concentration of methadone cannot be determined by this assay. The test is intended to

distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERRFOMANCE CHARACTERISTICS

A. Accuracy

The accuracy of the DRG® Rapid MTD test was evaluated in comparison to GC/MS at a cut-off of 300 ng/ml of methadone. One hundred and nineteen urine specimens with confirmed methadone concentrations were evaluated in this study. Borderline readings were recorded as negative. The results are summarized and presented below:

	(-)		(+)		
DRG® Rapid MTD Test	GC/MS Negative (less than -25% cut off)	Near cutoff negative (between –25% and c/o	Near cutoff positive (between c/o and +25%	GC/MS Positive (greater than +25% cut off)	Percent agreement with GC/MS
Positive	0	0	9	49	96.7
Negative	50	9	2	0	100
Total	50	9	11	49	

Positive % agreement: 96.7, Negative % agreement: 100.

Two specimens were found discrepant between the DRG® Rapid MTD and GC/MS method. When compared those data, 100% (2 out of 2) of the discrepancy specimens were found between cut-off and +25% cut-off concentration (300 – 375 ng/ml).

B. Sensitivity

The cut-off concentration (sensitivity level) of DRG® Rapid MTD test is determined to be 300ng/ml.

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 positive			No. of borderline #			No. of negative		
			1*	2*	3*	1*	2*	3*	1*	2*	3*
MTD	150	42							42	42	42
	225	42				23	27	23	19	15	19
	300	42	23	27	23	19	15	19			
	375	42	42	42	42						
	450	42	42	42	42						

D. Specificity

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The specificity for DRG® Rapid MTD 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 DRG® Rapid MTD test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.5 to 8.0 and 1.005 to 1.035.

The following substances were tested and confirmed not to interfere with DRG® Rapid MTD 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 DRG® Rapid MTD test which produced positive results when tested at levels equal or greater than the concentrations listed below:

The following table lists compounds that are detected by DRG® Rapid MTD Tests.

Compounds	Cut-off (ng/ml)	Cross reactivity (%)
Methadone	300	100
Doxylamine	50,000	0.6

The following compounds show negative rsults at concentration 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	Meperidine	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-Δ8-THC-9-COOH(10 µg/ml)
11-nor-Δ9-THC-9-COOH(10 µg/ml) Thioridazine Trifluoperazine
Tyramine Tryptophan

REFERENCES

1. Urine testing for drugs of abuse, NIDA Research Monograph 73 (1986)
2. Steven B. Karch, Drugs of abuse hand book, CRC Press, 1st. Ed. (1998)
3. Ray H. Liu and Bruce A. Goldberger, Handbook of workplace drug testing, AACC Press, Washington DC (1995)

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