Methadone Rapid Screen Test (RAP-3532)

Revised 16 Jun 2008

For the rapid determination of methadone in human urine

For *in vitro* diagnostic use only.

INTENDED USE

The DRG® International, Inc. Methadone Screen Test Card and Test Strip devices are in vitro diagnostic (IVD), qualitative, lateral flow, competitive, immunochromatographic, urinary screening devices intended for the rapid detection of methadone, its analogs and its metabolites (collectively termed: MED) at the Substance Abuse and Mental Health Services Administration (SAMHSA) cut-off level of 300 ng/ml. The tests are designed to obtain a visual, qualitative result and are intended for professional use only. They are not intended for quantitative results, nor for over-the-counter sale.
The DRG® Methadone Screen Test provides only preliminary analytical data. A more specific, alternative method is required to obtain a confirmed analytical result. SAMHSA has established gas chromatography / mass spectrometry (GC/MS) as the preferred confirmatory method. Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION OF TEST

Methadone is a synthetic narcotic/analgesic drug substitute, usually taken orally or by intravenous or intramuscular injections. Since dosing with methadone usually causes little euphoria but does appear to often result in physiologic dependency and tolerance, the drug has been widely prescribed for use in blocking opioid drug abuse. Methadone is most often used in maintenance programs as a substitute for heroin or other abused opioids, permitting patients to be functionally successful at work and in life while remaining in a drug rehabilitation program. Overdose with methadone can result in drowsiness and confusion, especially when potentiated by ethyl alcohol, or other CNS depressant drugs. Methadone is mainly metabolized to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP) in the liver, with excretion in urine within 24-48 hours. Methadone, its analogs and its metabolites are the primary urinary markers for detecting methadone use and abuse, ranging up to 1 to 5 ug/ml urine.
The DRG® Methadone Screen Tests are rapid, visual, lateral flow, competitive, immunochromatographic IVD assays for the qualitative detection of MED in human urine. These IVD screening tests are based on immunoassay principles and designed specifically for the analysis and identification of methadone, its analogs and its metabolites at a cut-off level of 300 ng/ml or higher, as set by SAMHSA.

PRINCIPLE OF THE PROCEDURE

The DRG® Methadone Screen Test is a competitive, IVD, immunochromatographic, urinary assay in which a chemically modified and bound methadone conjugate competes with urinary MED for limited, specific methadone antibody binding sites. Each test device contains a sample reaction unit, a pink-colored, antibody-colloidal gold conjugate unit pre-labeled with specific antibody, and a chromatographic membrane pre-coated with drug conjugate in the device test region.

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When free drug is absent from urine or at concentrations lower than the urinary detection cut-off level, the pink-colored, antibody-colloidal gold conjugate is free to bind to the immobilized drug conjugate pre-coated on the chromatographic membrane test region. Consequently, a pink-colored band will form in the test region indicating a negative result. When drug is present in urine at concentrations at or exceeding the detection cut-off level, it will bind to limited epitopes on the pink-colored, antibody-colloidal gold conjugate, completely competing with drug conjugate in the test region. In such cases, no band forms in the test region, indicating a positive result.
The DRG® Methadone Screen Test also provides a built-in process control. A pink-colored band should always appear in the control region, regardless of the presence of any urinary MED. This pink-colored control band verifies that: 1) sufficient urine volume was added, and 2) proper flow was obtained. If the control band is missing, the test was not performed correctly or failed to function correctly. In summary, negative urine will produce two pink-colored bands, one in the control region and one in the test region. Positive urine will produce one pink-colored band in the control region only.

REAGENTS AND MATERIALS SUPPLIED

- Test device with the following components: a sample reaction unit, a pink-colored, colloidal gold conjugate unit, and a chromatographic membrane unit.
- A disposable sample pipette comes with the test card.
- Test instructions

MATERIALS REQUIRED BUT NOT PROVIDED

- Urine sample collection containers
- Timer or clock

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.
Avoid cross contamination of urine samples by using a new sample collection container and pipette for each urine sample.
Urine specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container.
Do not use a test device beyond the expiration date imprinted on the outside of the foil pouch.

STORAGE AND STABILITY

The test devices supplied can be stored at room temperature or refrigerated (2-28°C) and will remain stable until the expiration date. **Do Not Freeze.**

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SAMPLE COLLECTION AND PREPARATION

The fresh urine samples should be collected in a clean, dry container such that same-day testing may be performed. Urine specimens may be refrigerated at 2-8°C for 48hours, or frozen at -20°C for assaying at a later date. Specimens that have been refrigerated or frozen must be equilibrated 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.

ASSAY PROCEDURE

Do not open foil pouch until ready to begin testing. Refrigerated test device should be allowed to equilibrate to room temperature (15-28°C) before pouch is opened.
Remove the test device from the sealed foil pouch by tearing along the notch.
FOR TEST STRIP: Immerse the strips in urine with the arrow end pointing towards the urine. Do not introduce urine above the maximum level, as indicated by the arrows.
FOR TEST CARD: Draw the urine sample into the pipette and dispense four drops (approximately 0.2ml) into the sample well of test device.
Read the test result at five minutes.

IMPORTANT: In order to prevent an incorrect reading, do not read the test results after more than 10 minutes. After 10 minutes, the intensity of the colored lines may change or a new line may appear. To avoid confusion, discard the test device after interpreting the result.

INTERPRETATION OF RESULTS

Negative: Two pink-colored bands appear, one in the control region and one in the test region. A negative result indicates free drug is absent from urine or at concentrations lower than the detection cut-off level of the test.
Positive: One pink-colored band appears in the control region with no apparent band appearing in the test region. A positive result indicates free drug is present in urine at concentrations at or exceeding the detection cut-off level of the test.
Invalid: No band appears in the control region, or a pink-colored band appears in the test region only. An invalid test result may be due to improper assay procedures or damage to the device. If results are invalid, the assay is inconclusive and the specimen should be re-tested using a new test device.
Note: The test band intensity may be weaker or stronger than that of the control band.

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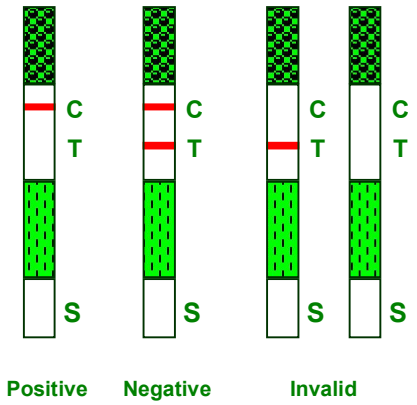
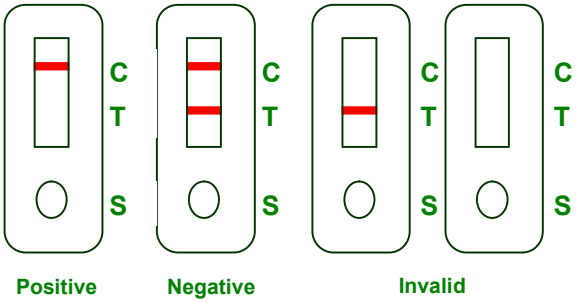
TEST STRIPS



TEST CARDS



PROCESS AND QUALITY CONTROL

Good laboratory practice requires use of control materials that ensure proper test device performance and reliability. Quality control standards are available for the validation of device functionality from commercial sources such as BioRad, Alltech and Biopool. To test the quality of control standards, use the assay procedure for testing urine samples. The SAMHSA recommended guidelines for drugs of abuse screening test devices indicate that controls should contain the drug of abuse analyte at levels at least 25% above cut-off values.

AFTER TESTING

Urine specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container. Residual urine should be disposed of in a medically approved manner after the completion of all testing, including the confirmatory assay.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The DRG® Methadone Screen Tests have been designed for detection of Methadone in urine at the detection sensitivity of 300 ng/ml, as suggested for immunoassay methods by SAMHSA. In sensitivity studies performed, samples with concentrations of methadone equal to or higher than 300 ng/ml were identified as positive results for all samples. Thus, the cut-off level of the DRG® Methadone Screen Test was determined to be 300 ng/ml for both the Test Card and Test Strip devices.

PRECISION

In order to determine the precision of both the DRG® Methadone Screen Test Card and Test Strip devices, both within-lot and inter-lot reproducibility studies were performed. Results of the within-lot reproducibility studies clearly showed excellent repeatability for all positive and negative urine samples using one lot of DRG® Methadone Screen Test Card devices and one of Test Strip devices. The results of inter-lot reproducibility studies clearly demonstrated that there was no appreciable inter-lot variation when testing both positive and negative samples across three different lots of DRG® Methadone Screen Test Card and Test Strip devices.

SPECIFICITY STUDY

Device specificity was tested against compounds related to, or not associated with, methadone, prepared in drug-free, normal human urine. The following compounds produced positive results in the test device at concentrations at or greater than the concentrations listed below.

Methadone	300 ng/ml
Methadol	1000 ng/ml
2-Ethylidene-1,5-dimethyl-3-diphenylpyrolidine (EDDP)	10,000 ng/ml
Ketamine ("Special K")	10,000 ng/ml
Nor-1etyl-Methadol (Nor-LAAM)	25,000 ng/ml
D-lysergic Acid Diethylamide(LSD)	50,000 ng/ml
Sodium Dodecyl Sulfate (SDS)	50,000 ng/ml
α-Acetyl-N,N-Dinormethadol(Dinor-LAAM)	50,000 ng/ml
1-α-Acetyl-Methadol (LAAM)	50,000 ng/ml

The following compounds were found not to have any impact on negative results when tested at levels of up to 100 µg drug/ml (100,000 ng drug/ml).

Acetaminophen (N-Acetyl-p-aminophenol)
Acetone
Albumin
Amitriptyline
Ampicillin
Aspartame (Asp-Phe Methyl Ester)
Aspirin (Acetylsalicylic Acid)
Atropine
Benzocaine (Ethyl p-Aminobenzoate)
Bilirubin
Caffeine
Chloroquine
(+)-Chlorpheniramine
(+/-)-Chlorpheniramine

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Creatine
Dexbrompheniramine ([+]-Brompheniramine)
Dextromethorphan
4-Dimethylaminoantipyrine
Dopamine (3-Hydroxytyramine)
Erythromycin
Ethanol
Furosemide
Glucose
Guaiacol glyceryl ether
Hemoglobin
Imipramine
(+/-)-Isoproterenol
(1R,2S)-(-)-N-Methyl-Ephedrine
(+)-Naproxen
Oxalic Acid
Penicillin-G (Benzylpenicillin)
Pheniramine
Phenothiazine (Thiodiphenylamine)
Quinidine
Ranitidine
Sulindac
Thioridazine
Trifluoperazine
Trimethobenzamide
Tyramine
Vitamin C (L-Ascorbic Acid)

ASSAY COMPARISONS & EQUIVALENCY

Accuracy and equivalency comparisons of both the DRG® Methadone Screen Test Card and Test Strip were evaluated against 163 individual in-house laboratory urine samples, as well as against 253 individual external SAMHSA-certified clinical laboratory urine samples. The results have been tabulated below.

Table 1. DRG® Methadone Card vs. Emit® II

DRG®	EMIT II (+)	EMIT II (-)	Row Totals
(+)	160	1	161
(-)	3	252	255
Col. Totals	163	253	416

When compared to Emit® II, the percent agreement with DRG® Methadone Screen Test Card positive samples was 160/163 or 98.2%. Negative samples recovered at 252/253 or 99.6%, while the overall relative accuracy obtained was 412/416 or 99.0%.

Table 2. DRG® Methadone Strip vs. Emit® II

DRG®	EMIT II (+)	EMIT II (-)	Row Totals
(+)	160	1	161
(-)	3	252	255
Col. Totals	163	253	416

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When compared to Emit® II, the percent agreement with DRG® Methadone Screen Test Strip positive samples was 160/163 or 98.2%. Negative samples recovered at 252/253 or 99.6%, while the overall relative accuracy obtained was 412/416 or 99.0%.

Table 3. DRG® Card vs. GC/MS

DRG®	GC/MS (+)	GC/MS (-)	Row Totals
(+)	124	2	126
(-)	0	175	175
Col. Totals	124	177	301

When compared to the GC/MS data, the relative sensitivity or percent agreement of DRG® Methadone Screen Test Card positive samples with the external clinical study was 124/124 or 100%. Negative samples recovered a relative specificity of agreement of 175/177 or 98.9%. Finally, the overall relative accuracy obtained was 299/301 or 99.3%.

Table 4. DRG® Methadone Strip vs. GC/MS

DRG®	GC/MS (+)	GC/MS (-)	Row Totals
(+)	124	2	126
(-)	0	175	175
Col. Totals	124	177	301

When compared to the GC/MS data, the relative sensitivity or percent agreement of DRG® Methadone Screen Test Strip positive samples with the external clinical study was 124/124 or 100%. Negative samples recovered a relative specificity of agreement of 175/177 or 98.9%. Finally, the overall relative accuracy obtained was 299/301 or 99.3%.

LIMITATIONS OF PROCEDURE

1. This diagnostic test is designed for the medical or forensic in vitro detection of methadone, its analogs and its metabolites in human urine.
2. This diagnostic test provides a qualitative screening for methadone, its analogs and its metabolites (MED) in urine. It is not to be used for quantitative determination of MED concentration in urine. A positive result indicates only the presence of MED and should be confirmed by GC/MS. It does not indicate intoxication.
3. There is a possibility of false positive results due to user error or the presence of interfering factors in urine.
4. See “SPECIFICITY STUDY” for substances that produce positive results at high concentrations and those substances which do not interfere with test performance.

5. Adulterants, such as bleach or other strong oxidizing agents, may produce erroneous test results when added to urine specimens, regardless of the analysis method used. If an adulteration is suspected, a fresh urine specimen should be used.

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