QuickScreen™ Methadone Test

Revised 26 Jan. 2006 **IVD**

Intended Use
The QuickScreen One Step Methadone Screening Test is a rapid, qualitative immunoassay for the detection of Methadone in urine. The cutoff concentration for this test is 300 ng/ml. This assay is intended for professional use.

This test provides only a preliminary test result. A more specific alternate testing method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Other confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test results.

Summary and Explanation of the Test
Methadone is a synthetic opioid, clinically available in the US since 1947. It acts on the central nervous system and cardiovascular systems producing respiratory and circulatory depression. It also produces meiosis and increases the tone of smooth muscle in the lower gastrointestinal tract while decreasing the amplitude of contractions. Methadone is metabolized in the liver by N-demethylation to form the metabolites 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) and 2-Ethyl-5-Methyl-3,3-Diphenylpyrrolidine (EMDP). These and the parent drug undergo hydroxylation with subsequent conjugation with glucuronid acid. All are excreted in bile and are the major products measured after methadone administration. Excretion rates vary from 5 to 50% of a dose in 24 hours, dependent on urine volume, pH, dosage and metabolism rate. Methadone is used clinically for treatment of severe pain and in treatment programs for morphine and heroin addiction.

Urine-based screening tests for drugs of abuse range from complex analytical procedures to simple immunoassay tests. The sensitivity and rapidity of immunoassays have made them the most accepted method of preliminary screening for drugs of abuse in urine. This allows the laboratory to eliminate a large number of negative specimens and focus on the smaller number of initially positive samples.

Principles of the Procedure
QuickScreen™ One Step Methadone Screening Test is a competitive immunoassay that is used to screen for the presence of Methadone in urine. It is a chromatographic absorbent device in which drug or drug metabolites in a sample compete with drug/protein conjugate immobilized on a porous membrane for a limited number of antibody/dye conjugate binding sites. The test device employs a unique combination of monoclonal and polyclonal antibodies to selectively identify Methadone in urine with a high degree of confidence.

In the procedure, urine is added to the test device's "SAMPLE" well with the aid of a plastic transfer pipette. The urine is absorbed into the device by capillary action, mixes with the antibody / dye conjugate, and flows across the pre-coated membrane. **When sample Methadone levels are below 300 ng/ml** (the detection sensitivity of the test), antibody/dye conjugate binds to the drug/protein conjugate immobilized in the Test region (**T**) of the device. This produces a colored Test Band that, *regardless of its intensity*, indicates a negative result.

When sample Methadone levels are at or above 300 ng/ml, the free drug in the sample binds to the antibody/dye conjugate, preventing the antibody/dye conjugate from binding to the drug/protein conjugate immobilized in the Test Region (**T**) of the device. This prevents the development of a distinct colored band, indicating a potentially positive sample.

In either case, a colored Control Band is produced in the Control Region (**C**) by a non-specific antibody-dye/conjugate reaction. This band serves as a built-in quality control device that demonstrates antibody recognition and reactivity as well as confirmation that the test result is valid.

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Reagents & Materials Supplied

1. **50 Test Devices:**
   a) Monoclonal anti-Methadone antibody/colloidal gold conjugate in a protein matrix containing 0.1% sodium azide coated in the sample path
   b) Methadone derivative/protein conjugate immobilized as a line in the test region
   c) Goat anti-mouse antibody immobilized as a line in the control region
2. **Directional Insert**

Warnings & Precautions

1. FOR IN VITRO DIAGNOSTIC USE ONLY.
2. For professional use only.
3. Urine samples have the potential to be infectious. Follow Universal Precautions for proper handling and disposal methods.
4. Do not use this kit beyond its expiration date.
5. This method has been established using urine only. Other fluids have not been evaluated.
6. Do not reuse the Test Device.

Storage and Handling Requirement
Store at room temperature (15-28°C); do not freeze. Refer to the expiration date for stability.

Sample Collection and Preparation
A fresh urine sample should be collected in a clean, dry plastic or glass container, unused and without preservatives. Testing requires only a small volume (1-2 ml) of urine in the sample container. If required by your procedure, aliquot a portion of urine into a second container for later confirmation of results. If not required, dispose of all but 1-2 ml of urine and save the remainder for the QuickScreen test.

Samples may be tested immediately or stored for up to 48 hours at 2 to 8° C. For longer storage, freeze samples at -20° C or below.

Assay Procedure

Preparation
1. Confirm that all samples and test components are at room temperature (15-28°C) before testing.
2. Do not break the seal on the foil pouch until you are ready to perform the test.

Testing
1. Open the foil pouch at the notch and remove the test device and transfer pipette. Take care not to touch the exposed membrane. Place the device on a clean, level surface.
2. Hold the dropper vertically and dispense 4 full drops of urine into the "SAMPLE" well. Wait 5 seconds between adding each drop.
3. Read the results immediately at (10) minutes.

4. Attention: Results read after 10 minutes have elapsed and should be considered invalid.

Interpretation of Test Result

Negative: A Negative result is indicated by two (**2**) colored bands, one in the control region (**C**) and one in the test region (**T**). This result indicates a Methadone level that is below the detection sensitivity of 300 ng/ml.

Positive: A Positive result is indicated when only one (**1**) colored band appears in the Control Region (**C**) and no band appears in the Test Region (**T**). This result indicates a Methadone level that is at or above the detection sensitivity of 300 ng/ml.

Invalid: A test must be considered invalid if no bands appear or if a band appears in the test region without a control band. The presence of a control band is necessary to confirm assay performance.

Quality Control

An internal procedural control line has been incorporated into the test device to help ensure proper kit performance and reliability. However, using external controls is recommended. Positive and negative controls, within 25% of the cutoff concentration should produce the expected result. For positive controls, only one (**1**) colored band will appear in the Control Region (**C**), and no band in the Test Region (**T**). For negative controls, two (**2**) colored bands will appear, one in the Control Region (**C**) and one in the Test Region (**T**).

Limitations of the Procedure

1. The possibility exists that substances and factors not described in this directional insert may interfere with the test, causing false results (e.g. technical or procedural error).
2. This test has been developed for testing urine samples only. The performance of this test using other specimens has not been substantiated.
3. Adulterated urine samples may produce erroneous results.
4. Strong oxidizing agents such as bleach (hypochlorite) can oxidize drug analytes. If a sample is suspected of being adulterated, obtain a new sample.
5. All positive samples must be confirmed by another method. Gas chromatography/mass spectrometry (GC/MS) is the method of choice to confirm the presence and concentration of a drug in urine.
6. This test is a qualitative, competitive screening assay. It is not designed to determine the quantitative concentration of drugs or the level of intoxication.
7. Because the QuickScreen is a competitive assay, no prozone effect is present.
8. Occasionally, samples containing target drug concentrations below the cut-off sensitivity for the test may produce a positive result.
9. Point-of-care testing data is not currently available.

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Performance Characteristics

Sensitivity (Cutoff) : The QuickScreen™ Methadone Test detects Methadone at a cutoff concentration equivalent to 300 ng/ml of Methadone. 80 urine samples were assayed in a controlled study, with 95% of samples within 25% of the cutoff correctly identified and greater than 99% of all other samples correctly identified.

Sample, n	Concentration Range (ng/ml of Methadone)	Observed vs Expected Results	Sensitivity	The technician reported that 3 samples all within 13% of the cutoff at 262.5, 278.6 and 296 ng/ml produced light or very light test band intensities. This is considered a negative result by the QuickScreen protocol. One sample within 1% of the cutoff at 299.6 ng/ml was reported as positive. NOTE: all remaining performance characteristics data was determined using the strip format.
20	0 to 150	20/20	>99%	
10	151 to 225	10/10	>99%	
10	226 to 300	10/10	>99%	
10	301 to 375	9/10	90%	
20	376 to 450	20/20	>99%	
10	451 to 600	10/10	>99%	

Kit Comparison – In an evaluation of 109 clinical urine samples at 3 separate laboratory sites, including 2 independent clinical laboratories, QuickScreen was compared to the Emit II Methadone test using a 300-ng/ml cutoff. The QuickScreen Methadone Test demonstrated an overall agreement of greater than 98% (107/109) when compared to the Emit II assay

Agreement		**Emit II Methadone**	
		Positive (49)	Negative (60)
QuickScreen	(+)	47	0
Methadone	(-)	2**	60

[1] Samples exhibited pronounced peaks in the ion windows for Methadone, however, compound(s) not identified.
[2] Two samples were reported as "borderline positive" (±) by technician. Faint test bands seen, but GC/MS negative.

**Resolution of Discrepant Results:

Sample	**QuickScreen**	**EMIT II**	**GC/MS**
X9475677D10	-	+	576 ng/ml
X930016D10	-	+	560 ng/ml

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Precision: Eight clinical urine pools ranging in concentration from 25 to 576 ng/ml were assayed once a day for twenty days using the QuickScreen Methadone Test. The results were interpreted individually by two technicians. Sample concentrations were confirmed by GC/MS analysis.

Clinical Sample	Concentration (ng/ml of Methadone)	Percent of Cutoff	Tech 1 Results	Tech 2 Results	Total Correct	Percent Correct
1	25	8.3	20/20 negative	20/20 negative	40/40	100
2	97	32	20/20 negative	20/20 negative	40/40	100
3	195	65	20/20 negative	20/20 negative	40/40	100
4	288	96	20/20 negative	20/20 negative	40/40	100
5	373	124	20/20 pos.	20/20 pos.	40/40	100
6	422	141	20/20 pos.	20/20 pos.	40/40	100
7	472	157	20/20 pos.	20/20 pos.		
8	576	192	20/20 pos.	20/20 pos.	40/40	100
		Totals	**160/160**	**160/160**	**320/320**	**100**

Cross-Reacting Substances: Structurally related compounds were prepared in normal human urine and tested for cross-reactivity with the QuickScreen Methadone Test. The results are expressed as the amount of compound capable of producing a result equivalent to 300 ng/ml of Methadone.

Compound	(-)-α-Acetylmethadol (LAAM)	(-)-α-Methadol	(±)-Methadone
Concentration	1,0 µg/ml	0,8 µg/ml	0.3 µg/ml

Interfering Substances

Extreme endogenous conditions of pH (4.5-8.5) and specific gravity (1.005-1.040) in normal human urine were found not to interfere with QuickScreen results. In addition, the following compounds were prepared in normal human urine and tested for interference with the QuickScreen™ Methadone Screening Test. Unless noted, the compounds were tested to 100 µg/ml with no interference observed.

Acetoacetic Acid – Acetone – *N*-Acetylprocainamide – Acetylsalicylic Acid (Aspirin) – Albumin – Alprazolam[(A)] – Amantadine – (+)-Amethopterin – Amikacin – *dl*-Aminoglutethimide – Aminopyrine – Amitriptyline – Amoxicillin – *d, dl* & *l*-Amphetamine – Ampicillin – Apomorphine – Aprobarbital - (-)-Arterenol – *l*-Ascorbic Acid (Vitamin C) – *d, dl & l*-Aspartic Acid, Atropine – Barbital - Barbituric Acid – Benzoic Acid – Benzoylecgonine – Benzphetamine – Benztropine Methane Sulfonate – Bilirubin – Bromazepam - Bromocriptine Mesylate – (+)-Brompheniramine – Butarbital - Butalbital - Butethal - Cannabidiol – Cannabinol - Carbamazepine – Cephalexin – Chloramphenicol – Chlordiazepoxide – Chloroquine – (+) & (±)-Chlorpheniramine – Chlorpromazine – Chlorpropamide – Chlorprothixene – Cimetidine – Clemastine – Clomipramine – Clonazepam – Clonidine – Cocaine – Codeine – (-)-Cotinine – Creatinine – Cyclizine – Cyclobenzaprine – Cyclosporin A - Cyproheptadine – (-)-Deoxyephedrine - Desipramine – Desmethyldiazepam – Dextromethorphan – 5-5-Diallylbarbituratic Acid - Diazepam – Diflunisal – Digoxin – Diphenhydramine – 4-Dimethyl-aminoantipyrine- Diphenoxylate – 5,5-Dipenylhydantoin – Disopyramide – Doxepin – Doxylamine – (+) & (-)-ψ-Ephedrine - (+),(±) & (-)-Ephedrine – (±) & (-)-Epinephrine – Erythromycin – Estriol – Estrone-3-Sulfate – Ethosuximide – Ethyl-*p*-Aminobenzoate – Ethylenediaminetetraacetic Acid – 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) – Ethylmorphine[B] - Fenfluramine – Fenoprofen - Fentanyl[B] - Flunitrazepam – Flurazepam – Furosemide –

Gentamicin – Gentisic Acid – *dl*-Glutethimide – Griseofulvin – Guaiacol Glyceryl Ester – Hemoglobin, Human – Heroin[B] - Hexobarbital – Hydrochlorothiazide – Hydrocodone – Hydromorphone – *dl*-*β*-Hydroxybutyric Acid – *o*-Hydroxyhippuric Acid – 5-Hydroxyindole-3-Acetic Acid – 5-Hydroxyindole-2-Carboxylic Acid – 11-Hydroxy-Δ^9-THC[(C)] – 3-Hydroxytyramine – Hydroxyzine – Imipramine – Indole-3-Acetic Acid – Indole-3-Butyric Acid – Indomethacin – (+)& (-)-Isoproterenol – Isoxsuprine – Kanamycin – Ketamine – Ketoprofen – Labetalol – Levorphanol – Lidocaine – Lithium Carbonate – (±)-Lorazepam – Lormetazepam – Lysergic Acid Diethylamide[D] - Medazepam – Melanin – Meperidine – Mephentermine – Meprobamate – Mescaline – *dl*-Methaneprine – (+)-Methamphetamine – Methaqualone – (S)-6-Methoxy-α-Methyl-2-Naphthaleneacetic Acid – 2-Methyl-3-(3,4-Dihydroxyphenyl)-*dl* & *l*-Alanine – (±)-3,4-Methylenedioxyamphetamine – (±)-3,4-Methylenedioxymethamphetamine – Methylphenidate –Methyprylon – Metoclopramide – (±)-Metoprolol – Morphine – Morphine-3-*β*-D-Glucuronide - Nafcillin – Nalorphine – Naloxone – Naltrexone – Naphazoline - α & β-Naphthaleneacetic Acid – Netilmicin – Niacinamide – Nialamide – Nicotinic Acid – Nifedipine – Nitrazepam – Nomifensine – Norcodeine – Nordoxepin[B] - Norethindrone - Normorphine[B] - 11-Nor-Δ^8 & Δ^9-THC-Carboxylic Acid[C] Nortriptyline – Noscapine – Nylidrin – Orphenadrine – Oxalic Acid – Oxazepam – Oxycodone – Oxymetazoline – Papaverine – Penicillin G – Pentazocine – Pentobarbital - Phencyclidine – Phenelzine – Pheniramine – Phenobarbital Phenothiazine – Phentermine – Phenylacetone – *l*-Phenylalanine – Phenylbutazone – *trans*-2-Phenylcyclopropylamine – *l*-Phenylephrine – (R)-(+)-α, (±)-α & β-Phenylethylamine – (±)-Phenylpropanolamine – Piroxicam – Potassium Chloride – Prazepam – Prednisolone – Primidone – Procainamide – Procaine – Prochlorperazine – Promazine – Promethazine – (+)-Propoxyphene – 2-Propylpentanoic Acid – Protriptyline – Pyrilamine – Quinidine – Quinine – Ranitidine – Riboflavin – Salicylic Acid – (-)-Scopolamine – Secobarbital - Sodium Chloride - Sulindac – Temazepam – Terbutaline – Tetracycline – Tetraethylthiuram Disulfide - Δ^8 & Δ^9-Tetrahydrocannabinol – Tetrahydrozoline – Thebaine – Theophylline – (±)Thiopental - Thioridazine – *cis*-Thiothixene Tobramycin – Triamterene – Triazolam[B] - Trifluoperazine – Triflupromazine – *dl*-Trihexyphenidyl – Trimethobenzamide – Trimethoprim – Trimipramine – Triprolidine – Tyramine – Urea – Uric Acid – Vancomycin – (±)-Verapamil – Zomepirac.

[A)] No intereference was observed when the compound was tested to 25 µg/ml.
[B] No interference was observed when the compound was tested to 10 µg/ml
[C] No interference was observed when the compound was tested to 5 µg/ml
[D] No interference was observed when the compound was tested to 2.5 µg/ml

Bibliography

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