DRI® Methadone Metabolite Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

REF 10018522 (3 x 18 mL Kit)
100115 (100 mL Kit)
100116 (500 mL Kit)

Intended Use
The DRI® Methadone Metabolite Assay is intended for the qualitative and semiquantitative determination of the presence of Methadone Metabolite, (2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine or EDDP), in human urine at cutoff 1000 ng/mL. The semiquantitative range of the assay is 31-2000 ng/mL. The assay provides a simple and rapid analytical screening procedure to detect methadone metabolite in human urine.

This assay provides only 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) is the preferred confirmatory method.[1,2] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary and Explanation of the Test
Methadone is a synthetic opiate that effectively suppresses the craving for heroin without the euphoric effects of heroin. Methadone is commonly used in treatment facilities to detoxify and maintain heroin addicts. Methadone treatment compliance is essential and can be effectively monitored by urine screening of methadone and its metabolite.

The mechanism of methadone metabolism is commonly understood. Once administered, methadone is quickly metabolized by the liver into normethadone by N-demethylation. Normethadone is rarely detected, because it readily dehydrates to form EDDP,[3,4] the primary metabolite of methadone. Further demethylation of EDDP forms 2-ethyl-5-methyl-3, 3-diphenyl-1-pyrroline (EMDP), the secondary metabolite of methadone, which is present in lower concentrations.[5]

Various immunoassay techniques are currently available for methadone compliance monitoring.[6,7] However, these tests measure the parent drug only (i.e., methadone) and are subject to "false positives" from addicts who add a portion of their methadone directly into the urine sample. As a result, confirmation of the presence of EDDP by thin layer chromatography (TLC) or gas chromatography (GC) is often required. Both TLC and GC methods[7] are laborious and subject to considerable interference. Determination of the presence of EDDP in urine with an immunoassay makes possible the widespread testing for compliance and rules out the possibility of adding Methadone to urine in clinics where unsupervised urine collections are permitted.[8]

The DRI Methadone Metabolite assay utilizes liquid ready-to-use reagents and calibrators.[9] The assay uses specific antibodies that can detect EDDP in human urine without cross-reactivity to the parent drug, methadone. The assay is based on competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH), and free drug from the urine sample for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH and causes a decrease in enzyme activity. This phenomenon creates a direct relationship between drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

Reagents
Antibody/Substrate Reagent (R1):
Contains mouse monoclonal anti-EDDP antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
Enzyme Conjugate Reagent (R2):
Contains EDDP derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

Additional Materials Required (sold separately):

REF	Kit Description
1664	DRI Negative Calibrator, 10 mL
1388	DRI Negative Calibrator, 25 mL
100117	DRI Methadone Metabolite 150 ng/mL Calibrator, 10 mL
100118	DRI Methadone Metabolite 300 ng/mL Calibrator, 10 mL
100120	DRI Methadone Metabolite 1000 ng/mL Calibrator, 10 mL
100122	DRI Methadone Metabolite 2000 ng/mL Calibrator, 10 mL
100200	MGC Primary DAU Control Set

⚠ Precautions and Warnings
Reagents used in the assay components contain ≤ 0.09% sodium azide. Avoid contact with skin and mucous membranes. Refer to SDS for additional precautions, handling instructions, and accidental exposure treatment.

BSA
The reagents contain ≤ 0.2% bovine serum albumin (BSA). Avoid contact with skin and mucous membranes. Avoid inhalation. May cause skin or inhaled allergic reaction. Refer to SDS for additional precautions, handling instructions, and accidental exposure treatment.

Reagent Preparation and Storage
The reagents are ready-to-use; no additional preparation is required. Reagents should be stored refrigerated. All assay components, opened or unopened, are stable until the expiration date indicated on their respective labels. Do not use the reagents beyond their expiration dates.

In the case of accidental spill, clean and dispose of material according to your laboratory's SOP, local, and state regulations.

In the case of damaged packaging on arrival, contact your technical support representative (refer to back page of this PI).

Specimen Collection and Handling
Collect urine specimens in plastic or glass containers. Fresh urine specimens should be used. *The Mandatory Guidelines for Federal Workplace Drug Testing Programs* recommend that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units.

Samples within a pH range of 3 to 11 are suitable for testing with this assay.

An effort should be made to keep pipetted samples free of gross debris. Centrifuge highly turbid specimens before analysis. Adulteration may cause erroneous results. If adulteration is suspected, obtain another sample and forward both specimens to the laboratory for testing.

Handle all urine specimens as if they were potentially infectious.

Assay Procedure
Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reaction accurately can be used to perform this immunoassay.

Refer to the specific application instructions of each analyzer for chemistry parameters before performing the assay.

Quality Control and Calibration[10]
Qualitative Analysis
For qualitative analysis of samples, use 1000 ng/mL calibrator as a cutoff level. The cutoff calibrator is used as reference for distinguishing "positive" from "negative" samples.

Semiquantitative Analysis
For semiquantitative analysis, use all calibrators.

Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Use controls near the cutoff calibrator to validate the calibration. Ensure that control results are within the established ranges as determined by laboratory procedures and guidelines. If results fall outside of the established ranges, assay results are invalid. All QC requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements. Currently, SAMHSA has made no recommendation regarding cutoff calibrator concentration. The State of California has recommended a cutoff concentration of 1000 ng/mL.

Results and Expected Values
Qualitative results
A sample that exhibits a change in absorbance value (ΔA) equal to or greater than the rate obtained with cutoff calibrator is considered positive. A sample that exhibits a change in absorbance value (ΔA) lower than the rate obtained with the cutoff calibrator is considered negative.

Semiquantitative results
A rough estimate of drug concentration in the samples can be obtained by running a standard curve with the appropriate calibrators and by quantitating samples off the standard curve. Sample results above the highest calibrator concentration in use should be diluted with negative urine and retested.

Limitations
1. A positive result from this assay indicates only the presence of EDDP and does not necessarily correlate with the extent of physiological and psychological effects.
2. It is possible that other substances and/or factors (technical or procedural), other than those investigated in the specificity study, may interfere with the test and cause false results.

Typical Performance Characteristics

Performance results obtained on the Hitachi 717 analyzer are shown below.[11] The results obtained in your laboratory may differ from these data.

Precision

The cutoff calibrator and controls (750 and 1250 ng/mL) were tested in qualitative (mA/min) and semiquantitative (ng/mL) modes using a modified NCCLS protocol. The cutoff calibrator and controls were tested in replicates of 6 and each test was run twice per day for 10 days.

Qualitative:

Calibrator/Control (n=20)	Within-run			Total-run		
	$\bar{x}$ (mA/min)	SD	%CV	$\bar{x}$ (mA/min)	SD	%CV
Negative Control (750 ng/mL)	426	2.7	0.6	426	3.1	0.7
Cutoff Calibrator (1000 ng/mL)	456	3.1	0.7	456	3.2	0.7
Positive Control (1250 ng/mL)	480	2.7	0.6	480	3.1	0.6

Semiquantitative:

Calibrator/Control (n=20)	Within-run			Total-run		
	$\bar{x}$ (mA/min)	SD	%CV	$\bar{x}$ (mA/min)	SD	%CV
Negative Control (750 ng/mL)	763	19.7	2.6	763	22.1	2.9
Cutoff Calibrator (1000 ng/mL)	1016	23.6	2.3	1016	25.7	2.5
Positive Control (1250 ng/mL)	1270	34.7	2.7	1270	36.8	2.9

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 31 ng/mL.

Accuracy

A total of 150 clinical specimens obtained from patients receiving methadone treatment were tested using the DRI Methadone Metabolite Assay and GC/MS. Comparison of results between the two methods produced a linear regression equation of y = 0.87x - 2.3 and a correlation coefficient (r) of 0.994 were obtained. Concordance (i.e., clinical agreement between both methods identifying a specimen as positive or negative) was greater than 95% between the subject device and the GC/MS. The data are presented below:

Qualitative

DRI Methadone Metabolite Assay

GC/MS	+	-
+	69	5**
-	0	76

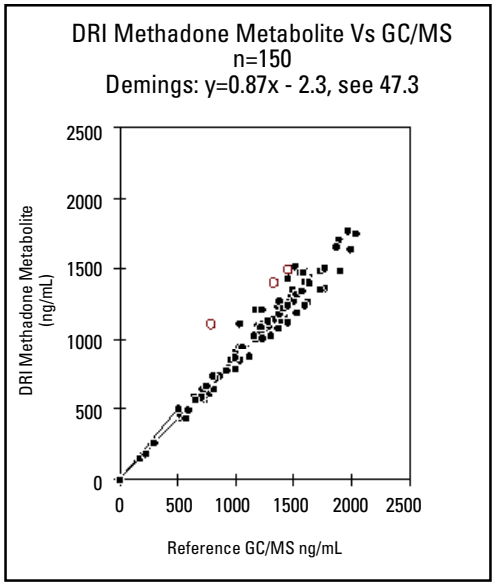
Semi-quantitative

DRI Methadone Metabolite Assay

GC/MS	+	-
+	69	7**
-	1†	73

** GC/MS results indicate that these samples contain1014-1208 ng/mL EDDP (i.e., the concentrations are approximately at the designated cutoff).

† GC/MS result indicates that the sample contains 790 ng/mL EDDP.



Specificity

The specificity of the assay was evalutated by testing parent drug and its metabolites. Other compounds that are commonly encountered in urine samples were also tested.

Methadone and its metabolites produced a negative result at the concentrations listed below.

Compound	Concentration (ng/mL)
Methadone	35,000,000
EMDP	200,000
LAAM-HCL	100,000
Nor-LAAM-HCL	100,000

Various compounds when tested at the concentrations listed below produced a negative result using 1000 ng/mL cutoff calibrator:

Compound	ng/mL	Compound	ng/mL
Acetaminophen	1,000,000	Ibuprofen	500,000
Acetylsalicylic acid	1,000,000	Ketamine	1,000,000
Amphetamine	1,000,000	Levothyroxine	500,000
Benzoylecgonine	1,000,000	Meperidine	1,000,000
Caffeine	100,000	d-Methamphetamine	100,000
Captopril	500,000	l-Methamphetamine	100,000
Chlordiazepoxide	100,000	Morphine	1,000,000
Cimetidine	500,000	Oxazepam	500,000
Cocaine	200,000	Phencyclidine	500,000
Codeine	1,000,000	Phenobarbital	1,000,000
Dextromethorphan	300,000	Phentermine	1,000,000
Diazepam	100,000	Promethazine	100,000
Diphenhydramine	500,000	Propoxyphene	1,000,000
Disopyramide	1,000,000	Ranitidine	500,000
Doxylamine	500,000	Salicyluric acid	500,000
Ephedrine	1,000,000	Secobarbital	1,000,000
Fluoxetine	500,000	11-Nor-Δ^9-THC-9-COOH	10,000

Interference

Endogenous and exogenous substances were studied for potential interference with the Methadone Metabolite assay. No interference was observed in urine samples containing the compounds up to the concentrations listed below. The pH of the urine sample was also studied for possible interference.

Compound	Concentration	Compound	Concentration
Acetaminophen	100 µg/mL	Glucose	3000 mg/dL
Acetone	1000 mg/dL	Hemoglobin	150 mg/dL
Ascorbic acid	1000 mg/dL	Human Serum Albumin	500 mg/dL
Aspirin	100 µg/mL	Ibuprofen	100 µg/mL
Caffeine	100 µg/mL	Oxalic acid	100 mg/mL
Creatinine	500 mg/dL	pH range	3-11
Ethanol	1 g/dL	Riboflavin	7.5 mg/dL
Galactose	10 mg/dL	Sodium Chloride	1 g/dL
γ-globulin	500 mg/dL	Urea	1.25 g/dL

References

1. "Urine Testing for Drug of Abuse". National Institute on Drug Abuse (NIDA) Research Monograph 73 (1986).
2. "Mandatory Guidelines for Federal Workplace Drug Testing Programs". National Institute on Drug Abuse. Federal Register Vol. 53, No 69, pp11970 (1988).
3. Pohland A, Boaz HE and HR Sullivan. Synthesis and Identification of Metabolites Resulting from the Biotransformation of d,-l-Methadone in Man and in Rat. J Med Chem 14: 194-197 (1971).
4. Baselt RC and LJ Casarett. Urinary Excretion of Methadone in Man. Clin Phrm Theap 13: 64-70 (1971).
5. Randall C. Baselt and Robert H. Cravey. Disposition of Toxic Drugs and Chemicals in Man. pp 472-475 4th Ed. Chemical Toxicology Institute. (1995).
6. Ferrara SD. Comparison of GLC-EMIT analysis for the Assay of Methadone and its Metabolite in Urine. Vet Hum Toxicology 21(suppl): 169-172 (1979).
7. Roerig DL et al. Radioimmunoassay Compared to Thin-Layer and Gas-Liquid Chromatography for Detecting Methadone in Human Urine. Clin Chem 22: 1915-1918 (1976).
8. Golman FR and CI Thistle. Diversion of Methadone: Illicit Methadone Use among Applicants to Two Metropolitan Drug Abuse program. Intl J Addictions, 13: 855-862 (1978).
9. Rubenstein KE, Schneider RS, and EF Ullman. "Homogenous Enzyme Immunoassay: A New Immunochemical Technique". Biochem Biophis Res Commun 47, 846, (1972).
10. Data on traceability are on file at Microgenics, a part of Thermo Fisher Scientific.
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