MDNII

cobas®

ONLINE DAT Methadone II

Drug abuse testing

Order information

REF	CONTENT		Analyzers on which **cobas c** pack can be used
03046702 190	ONLINE DAT Methadone II (200 tests)	System-ID 07 6722 0	COBAS INTEGRA 400 plus COBAS INTEGRA 800
03304671 190	Preciset DAT Plus I CAL 1-6 (6 × 5 mL)		
03304698 190	C.f.a.s. DAT Qualitative Plus (6 × 5 mL)		
04590856 190	C.f.a.s. DAT Qualitative Plus Clinical (3 × 5 mL)		
03312950 190	Control Set DAT I PreciPos DAT Set I (2 × 10 mL) PreciNeg DAT Set I (2 × 10 mL)		
04500873 190	Control Set DAT Clinical PreciPos DAT Clinical (2 × 10 mL) PreciNeg DAT Clinical (2 × 10 mL)		

English

System information

Test MD3S2, test-ID 0-322 for semiquantitative assay

Test MD3Q2, test-ID 0-222 for qualitative assay

Test MD3QC, test-ID 0-422 for qualitative assay, using C.f.a.s. DAT Qualitative Plus Clinical

Intended use

Methadone II (MDNII) is an in vitro diagnostic test for the semiquantitative and qualitative detection of methadone in human urine at a cutoff concentration of 300 ng/mL on COBAS INTEGRA systems. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program. Semiquantitative assays are intended to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas chromatography/mass spectrometry (GC/MS).

Methadone II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. GC/MS is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Methadone is a synthetic diphenylpropylamine used for detoxification and temporary maintenance of narcotic addiction, as well as to treat acute and chronic pain. Methadone has many of the pharmacologic properties of morphine, and its analgesic potency is similar. Unlike morphine, repeated administration causes marked sedative effects due to drug accumulation in the body. Methadone withdrawal syndrome is qualitatively similar to morphine, yet differs in that it develops more slowly, is less intense, and is more prolonged.[2] For these reasons, methadone is used in the management of narcotic dependence, hopefully eliminating the need for illicit opiate drugs. Overdoses of methadone are characterized by stupor, respiratory depression, cold and clammy skin, hypotension, coma, and circulatory collapse.[3]

Methadone is given intramuscularly for analgesic purposes and orally for methadone maintenance therapy. Following ingestion, the drug is well absorbed from the gastrointestinal tract and is widely distributed to the liver, lung, kidney, spleen, blood, and urine. The fact that methadone is highly bound to tissue protein may explain its cumulative effects.[4] Methadone is metabolized largely by mono- and di-N-demethylation. Spontaneous cyclization of the resulting unstable compounds forms the major metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). Both are hydrolyzed to some extent, with subsequent glucuronidation.[5,6] In maintenance patients, excretion of unchanged methadone can account for 5 to 50 % of the dose. Urinary pH affects the percentage of unchanged drug excreted, as does urinary volume, dose, and individual metabolism.[7,8]

Test principle

Kinetic interaction of microparticles in a solution (KIMS)[9,10] as measured by changes in light transmission.

In the absence of sample drug, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases. When a urine sample contains the drug in question, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.[11]

Reagents - working solutions

R1 Conjugate Reagent
Conjugated methadone derivative in buffer with BSA and 0.09 % sodium azide.

SR Antibody/Microparticle Reagent
Microparticles attached to methadone antibody (mouse monoclonal) in buffer with BSA and 0.09 % sodium azide.

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

Reagent handling

COBAS INTEGRA 400 plus analyzer

Mix all new (non-punctured) **cobas c** packs for 1 minute on a cassette mixer before loading on the analyzer. All in-use **cobas c** packs must also be mixed in the same manner at the beginning of each week (once a week).

COBAS INTEGRA 800 analyzer

Ready for use. After **cobas c** pack puncture, the analyzer automatically mixes the reagent for 1 minute and for half a minute during Begin of Day.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus analyzer	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 analyzer	
On-board in use at 8 °C	12 weeks

Do not freeze reagents. Reagents that have been frozen should be discarded.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[12]

For prolonged storage, freezing of the sample is recommended.

Centrifuge highly turbid specimens before testing.

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs.*[13]

Caution: Specimen dilutions should only be used as an estimation for GC/MS and are not intended for patient values. Dilution procedures, when used, should be validated.

Materials provided

See "Reagents – working solutions" section for reagents.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for urine

COBAS INTEGRA 400 plus test definition

	Semiquantitative	*Qualitative*
Measuring mode	Absorbance	Absorbance
Abs. calculation mode	Endpoint	Endpoint
Reaction mode	R1-S-SR	R1-S-SR
Reaction direction	Increase	Increase
Reaction start	SR	SR
Wavelength A	651 nm	651 nm
Test range	0-2000 ng/mL	0-3000
with postdilution	0-20000 ng/mL	
Postdilution factor	10 recommended[a)]	No
Calc. first/last	35/47	35/47
Unit	ng/mL	

a) For use when estimating concentration in preparation for GC/MS analysis.

Pipetting parameters		Diluent (H_2O)
R1	100 µL	10 µL
Sample	2 µL	8 µL
SR	35 µL	2 µL
Total volume	157 µL	

COBAS INTEGRA 800 test definition

	Semiquantitative	*Qualitative*
Measuring mode	Absorbance	Absorbance
Abs. calculation mode	Endpoint	Endpoint
Reaction mode	R1-S-SR	R1-S-SR
Reaction direction	Increase	Increase
Reaction start	SR	SR
Wavelength A	651 nm	651 nm
Test range	0-2000 ng/mL	0-3000
with postdilution	0-20000 ng/mL	
Postdilution factor	10 recommended[b)]	No
Calc. first/last	45/78	45/78
Unit	ng/mL	

b) For use when estimating concentration in preparation for GC/MS analysis.

Pipetting parameters		Diluent (H_2O)
R1	100 µL	10 µL
Sample	2 µL	8 µL
SR	35 µL	2 µL
Total volume	157 µL	

Calibration

Calibrators	*Semiquantitative application*
MD3S2, 0-322	Preciset DAT Plus I calibrators, CAL 1-5 0, 150, 300, 600, 2000 ng/mL *d,l*-methadone (DATS1, system-ID 07 6752 2)
	Qualitative applications
MD3Q2, 0-222	Preciset DAT Plus I calibrators, CAL 1 0 ng/mL *or* deionized water *and* Preciset DAT Plus I calibrators, CAL 3[c)] *or* C.f.a.s. DAT Qualitative Plus 300 ng/mL (DATQ1, system-ID 07 6744 1)
MD3QC, 0-422	Preciset DAT Plus I or II[d)] calibrators, CAL 1 0 ng/mL *or* deionized water *and* C.f.a.s. DAT Qualitative Plus Clinical 300 ng/mL (DATQ5, system-ID 07 6880 4)
	For qualitative applications, the cutoff value is assigned as 1000.

c) Do not use Preciset DAT Plus I, CAL 3 if calibrating the Opiates 300/2000 qualitative 2000 ng/mL assay (test OP2QL, test-ID 0-410)

d) Preciset DAT Plus II, CAL 1, while generally not required for the calibration of Methadone II, may be used as an alternative 0 ng/mL level for DATQ5, system-ID 07 6880 4.

Calibration mode	*Semiquantitative application*
	Logit/Log 4
	Qualitative applications
	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	COBAS INTEGRA 400 plus analyzer:
	Each lot, every 4 weeks, and as required following quality control procedures.
	COBAS INTEGRA 800 analyzer:
	Each lot, every 4 weeks, and as required following quality control procedures.

A calibration curve is generated using the calibrators. Calibrators must be placed from the highest concentration first to the lowest last on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: This method has been standardized against a primary reference method (GC/MS).

Quality control

Quality control	Control Set DAT I
	PreciPos DAT Set I (DAT1P, system-ID 07 6753 0)
	PreciNeg DAT Set I (DAT1N, system-ID 07 6754 9
	or
	Control Set DAT Clinical
	PreciPos DAT Clinical (DATCP, system-ID 07 6879 0)
	PreciNeg DAT Clinical (DATCN, system-ID 07 6878 2)

Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

Drug concentrations of Control Set DAT I and Clinical have been verified by GC/MS.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Results

COBAS INTEGRA systems report results with the following test flags:

Semiquantitative result reporting

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 300 ng/mL
<TEST RNG	Negative	< 0 ng/mL
>TEST RNG	Positive	> 2000 ng/mL
POS 300	Positive	≥ 300 ng/mL

Value ranges listed above are based on a cutoff value of 300 ng/mL.

Qualitative result reporting

Flag	COBAS INTEGRA	Value range
No flag	Negative	< 1000
<TEST RNG	Negative	< 0
>TEST RNG	Positive	> 3000
POS 1000	Positive	≥ 1000

Value ranges above are based on assigning the cutoff of 300 ng/mL a value of 1000.

Semiquantitative result reporting

The semiquantitation of preliminary positive results should only be used by laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS. It also permits the laboratory to establish quality control procedures and assess control performance.

Note: When using the post-dilution function (1:10 dilution), to ensure the sample was not over-diluted, the diluted result must be at least half the analyte cutoff value times 10. If the diluted result falls below half the analyte cutoff value times 10, repeat the sample with a smaller dilution. A dilution that produces a result closest to the analyte cutoff is the most accurate estimation. To estimate the preliminary positive sample's concentration, multiply the result by the appropriate dilution factor. Dilutions should only be used as an estimation for GC/MS.

Limitations - interference

See the "Specific performance data" section of this document for information on substances tested with this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A preliminary positive result with this assay indicates the presence of methadone in urine. It does not measure the level of intoxication.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Interfering substances were added to drug free urine at twice the concentration listed below. These samples were then spiked to 300 ng/mL using a 600 ng/mL methadone stock solution. Samples were tested and the following results were obtained.

Substance	Concentration tested	% Methadone Recovery
Acetone	1 %	98
Ascorbic Acid	1.5 %	100
Bilirubin	0.25 mg/mL	86
Creatinine	5 mg/mL	100
Ethanol	1 %	99
Glucose	2 %	101
Hemoglobin	0.1 g/L	101
Hemoglobin	1 g/L	98
Hemoglobin	7.5 g/L	100
Human Albumin	0.025 %	101
Human Albumin	0.05 %	103
Human Albumin	0.5 %	101
Oxalic Acid	2 mg/mL	102
Sodium Chloride	0.5 M	97
Sodium Chloride	1 M	95
Urea	6 %	103

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined in an internal protocol using a series of methadone calibrator and controls in replicates of 20, once a day, for 5 days. The following results were obtained on a COBAS INTEGRA 700 analyzer.

Semiquantitative precision

Repeatability	Mean ng/mL	SD ng/mL	CV %
Level 1 (225 ng/mL)	238	6	2.4
Level 2 (300 ng/mL)	305	6	2.0
Level 3 (375 ng/mL)	374	8	2.2

Intermediate precision	Mean ng/mL	SD ng/mL	CV %
Level 1 (225 ng/mL)	228	8	3.5
Level 2 (300 ng/mL)	301	8	2.5
Level 3 (375 ng/mL)	377	8	2.2

Qualitative precision

Cutoff (x)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Lower detection limit of the test

15 ng/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying

2 standard deviations above that of the zero calibrator (zero calibrator + 2 SD, repeatability, n = 21).

Accuracy

100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel by another technology, were evaluated for methadone on a COBAS INTEGRA 700 analyzer. All 100 clinical samples were negative relative to the 300 ng/mL cutoff.

55 urine samples, obtained from clinical laboratories where they screened preliminary positive by a commercially available enzyme immunoassay and confirmed positive for methadone by GC/MS, were also evaluated on a COBAS INTEGRA 700 analyzer. All 55 samples were positive with the COBAS INTEGRA Methadone II assay relative to the 300 ng/mL cutoff.

In addition, 10 samples were diluted to a methadone concentration of 75-100 % of the cutoff concentration; and 10 samples were diluted to a methadone concentration of 100-125 % of the cutoff concentration.

Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from the diluted positive urine samples. The following results were obtained with Methadone II on a COBAS INTEGRA 700 analyzer relative to the GC/MS values.

Methadone II Clinical Correlation (Cutoff = 300 ng/mL)

COBAS INTEGRA 700 analyzer		Negative samples	GC/MS values (ng/mL)		
			Near cutoff		
			225-241	310-375	470-10410
	+	0	0	10	55
	–	100	10	0	0

Analytical specificity

The specificity of the COBAS INTEGRA Methadone II assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to the 300 ng/mL methadone assay cutoff. Caution should be taken when interpreting results of patient samples containing structurally related compounds having greater than 0.5 % cross-reactivity.

Compound[e)]	Approximate ng/mL equivalent to 300 ng/mL of methadone	Approximate percent cross-reactivity
Hydroxymethadone	2571	11.7
Cyamemazine	5229	5.7
Methotrimeprazine (Levomepromazine)	6244	4.8
Vortioxetine	8524	3.5
Lu AA34443	2072	14
Thiothixene	24268	1.2
Chlorpromazine	26019	1.2
Promazine	92857	0.3
Clomipramine	94767	0.3
Thioridazine	109091	0.3
Chlorprothixene	168539	0.2
Promethazine	168539	0.2
l-α- methadol	215827	0.1
Trimipramine	229008	0.1
Cyclobenzaprine	256410	0.1
Orphenadrine	258621	0.1
l-α-acetylmethadol HCl (LAAM)	291262	0.1
Diphenhydramine	291262	0.1
Perphenazine	315789	0.1
Meperidine	333333	0.1
Imipramine	361446	0.1
Cyproheptadine	410959	0.1
Doxepin	428571	0.1

e) Intended compounds are metabolites of the preceding drug.

Additionally, the following compounds were tested at a concentration of 100000 ng/mL in pooled human urine and shown to have cross-reactivity values of less than 0.05 %.

Amitriptyline	EMDP
Benzphetamine	Fluoxetine
Carbamazepine	Maprotiline
Chlorpheniramine	Mianserin
Desipramine	Nordoxepin
Dextromethorphan	Nortriptyline
Disopyramide	*d*-Propoxyphene
Doxylamine	Protriptyline
EDDP	*d,l* -Verapamil

Specimens from Seroquel (quetiapine fumarate) users have screened positive for methadone.

Drug interference

The following compounds were added to aliquots of pooled normal human urine at a concentration of 100000 ng/mL. None of these compounds gave values in the assay that were equal to or greater than 0.2 % cross-reactivity.

Acetaminophen	Lidocaine
Acetylsalicylic acid	LSD
Aminopyrine	MDA
Amobarbital	MDMA
d -Amphetamine	Melanin
l -Amphetamine	*d*-Methamphetamine
Ampicillin	*l*-Methamphetamine
Ascorbic acid	Methaqualone
Aspartame	Methylphenidate
Atropine	Methyprylon
Benzocaine	Morphine sulfate
Benzoylecgonine	Naloxone
(cocaine metabolite)	Naltrexone
Butabarbital	Naproxen
Caffeine	Niacinamide
Calcium hypochlorite	Nicotine
Chlordiazepoxide	Nordiazepam
Chloroquine	Norethindrone
Cocaine	*l*-Norpseudoephedrine
Codeine	Oxazepam
Cotinine	Penicillin G
Diazepam	Pentobarbital
Diphenylhydantoin	β-Phenethylamine

Dopamine	Phencyclidine
Ecgonine	Phenobarbital
Ecgonine methyl ester	Phenothiazine
Ephedrine	Phentermine
d-Ephedrine	Phenylbutazone
l-Ephedrine	Phenylpropanolamine
Epinephrine	*d*-Phenylpropanolamine
Erythromycin	Procaine
Estriol	*d*-Pseudoephedrine
Fenoprofen	*l*-Pseudoephedrine
Furosemide	Quinidine
Gentisic acid	Quinine
Glutethimide	Secobarbital
Guaiacol glycerol ether	Sulindac
Haloperidol	Tetracycline
Hydrochlorothiazide	Δ^9 THC-9-carboxylic acid
Ibuprofen	Tetrahydrozoline
Isoproterenol	Trifluoperazine
Ketamine	Tyramine

The cross-reactivity for Tramadol, at a concentration of 87278 ng/mL is 0.3 %. The cross-reactivity for Ofloxacin, at a concentration of 220000 ng/mL, was tested with Methadone II. The result obtained was 0.2 %.

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

References

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13 Mandatory Guidelines for Federal Workplace Drug Testing Programs. Fed Regist 2008 Nov 25;73:71858-71907.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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