MDN2

cobas®

ONLINE DAT Methadone II

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058008190	08058008500	ONLINE DAT Methadone II (850 tests)	System-ID 2088 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

Serum/plasma		
03304671190	Preciset DAT Plus I CAL 5 (1 x 5 mL)	Code 20435
07978766190	Serum DAT Control Low (ACQ Partner Channel*)	
07978740190	Serum DAT Control High (ACQ Partner Channel*)	
08063494190	NaCl Diluent 9 % (123 mL)	System-ID 2906 001

*Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.

Urine		
03304671190	Preciset DAT Plus I CAL 1-6 (6 x 5 mL)	Codes 20431-20436
03304698190	C.f.a.s. DAT Qualitative Plus (6 x 5 mL)	Code 20698
04590856190	C.f.a.s. DAT Qualitative Plus Clinical (3 x 5 mL)	Code 20699
03312950190	Control Set DAT I PreciPos DAT Set I (2 x 10 mL) PreciNeg DAT Set I (2 x 10 mL)	
04500873190	Control Set DAT Clinical PreciPos DAT Clinical (2 x 10 mL) PreciNeg DAT Clinical (2 x 10 mL)	

English

System information

MDQ3S: ACN 20883 (Serum/plasma): for qualitative assay, 300 ng/mL

MD3Q0: ACN 20880 (Urine): for qualitative assay, 300 ng/mL

MD3S0: ACN 20881 (Urine): for semiquantitative assay, 300 ng/mL

MD3QC: ACN 20882 (Urine): for qualitative assay, 300 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

MD3-QP: ACN 20884 (Urine): for qualitative assay, 300 ng/mL; using C.f.a.s. DAT Qualitative Plus

Intended use

Application in urine

Methadone II (MDN2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of methadone in human urine on **cobas c** systems at a cutoff concentration of 300 ng/mL. 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.

Application in serum and plasma*

**not available in all countries*

The ONLINE DAT II assay for Methadone is an in vitro diagnostic test for the qualitative detection of methadone in human serum and plasma on **cobas c** systems at a cutoff concentration of 300 ng/mL. The assay provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC-MS) or liquid chromatography coupled with tandem mass spectrometry (LC-MS/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

Detection of methadone in human serum, plasma and urine with this assay is used as an aid in monitoring adherence to treatment in patients under addiction programs and/or under pain treatment and for presumptive testing of illicit use of methadone in individuals with suspected exposure.

Methadone belongs to the fully synthetic opioids. It can be administered orally or intravenously and is used for detoxification and temporary maintenance of opioid use disorder, as well as treatment of acute and chronic pain.[2,3] Methadone is also subject of abuse, in this context withdrawal syndrome is qualitatively similar to morphine, yet it differs in that it develops more slowly, is less intense, and is more prolonged.[2] Methadone has a high bioavailability, a half-life of 28 h allowing a single dose daily.[4] Similar as morphine, methadone has affinity not only for the mu-opioid receptor (MOR) but also for the delta-opioid receptor (DOR). This might explain its utility in patients whose pain no longer responds to other opioids.[3] However, unlike morphine, repeated administration causes marked sedative effects due to drug accumulation in the body based on tissue protein binding.[5] It is distributed to the liver, lung, kidney, brain, gut, muscle, and urine.[6]

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.[7,8] In opioid dependent patients, excretion of unchanged methadone can account for 5-50 % of the dose. Urinary pH affects the percentage of unchanged drug excreted, as does urinary volume, dose, and individual metabolism.[9,10]

Clinical urine opioid drug testing is done to monitor adherence to methadone treatment and methadone is detectable from 1.5 to 3 days after administration.[11,12] To confirm that the patient has taken methadone and is not simply adding it to a urine specimen, the test for the methadone metabolite, EDDP, can be ordered.[11] Because many drugs are cleared from the blood rapidly, testing of blood or its components (serum) has short periods of detection.[13] Measurement in serum or plasma is an acceptable alternative for the detection of methadone in pain management patients with end-stage renal failure.[14]

In the context of drug screening, samples that test negative on initial screening tests can be reported as negative and disposed of as planned. Otherwise, depending on the situation, presence of the drug indicated by a

positive screening result may need to be confirmed using a suitable confirmatory technique (e.g., GC-MS or LC-MS).[12,13,14,15]

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[16,17] 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 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.[18]

Reagents - working solutions

R1 Conjugated methadone derivative; buffer; bovine serum albumin; 0.09 % sodium azide

R2 Microparticles attached to methadone antibody (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	26 weeks

Do not freeze.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum: Serum tubes with and without separating gel.
Plasma: K_2- or K_3-EDTA, lithium heparin.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability in serum:	5 days capped at 15-25 °C
	14 days capped at 2-8 °C
	6 months capped at -20 °C (± 5 °C)

Specimens can be repeatedly frozen and thawed up to 3 times.

Invert thawed specimens several times prior to testing.

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.[19]

For prolonged storage, freezing of the sample is recommended.[19]

Freeze only once.

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*.[20]

Centrifuge highly turbid specimens or samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC-MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

	Qualitative
Reporting time	10 min
Wavelength (sub/main)	– /546 nm
Reagent pipetting	
R1	59 µL
R2	26 µL
Sample volumes	*Sample*
300 ng/mL cutoff	
Normal	2.3 µL
Decreased	2.3 µL
Increased	2.3 µL

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Application for urine

Test definition

	Semiquantitative	Qualitative	
Reporting time	10 min	10 min	
Wavelength (sub/main)	– /546 nm	– /546 nm	
Reagent pipetting		Diluent (H_2O)	
R1	59 µL	–	
R2	26 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	2.3 µL	–	–

Decreased	2.3 µL	–	–
Increased	2.3 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Serum/plasma

Qualitative application

Calibrator	*300 ng/mL cutoff assay* S1: Preciset DAT Plus I, CAL 5, 2000 ng/mL with automatic pre-dilution
Cutoff Calibrator	A value of "0" is encoded in the e-barcode in order to ensure flagging of positive samples with >Test and negative absorbance values for negative samples.
Calibration K factor	The K factor of -1000 is predefined in the application settings.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 84 days - as required following quality control procedures

Urine

Semiquantitative application

Calibrators	*300 ng/mL cutoff assay* S1-5: Preciset DAT Plus I, CAL 1-5 0, 150, 300, 600, 2000 ng/mL
Calibration mode	Non-linear
Calibration frequency	Full calibration - after reagent lot change - every 12 weeks on-board - as required following quality control procedures

Qualitative application

Calibrators	*300 ng/mL cutoff assay* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Plus Clinical, Preciset DAT Plus I, CAL 3 300 ng/mL
Cutoff Calibrator	A value of "0" is encoded in the e-barcode in order to ensure flagging of positive samples with >Test and negative absorbance values for negative samples.
Calibration K factor	The K factor of -1000 is predefined in the application settings.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 12 weeks on-board - as required following quality control procedures

The drug concentration of the calibrator has been verified by GC-MS.

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: These methods have been standardized against a primary reference method (GC-MS).

Quality control

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 and the high and low controls have been verified by GC-MS or LC-MS/MS.

The control intervals and limits should be adapted to each laboratory’s individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

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

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between preliminary positive and negative samples. Samples producing a positive or “0” absorbance value are considered preliminary positive. Preliminary positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

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.

cobas c systems automatically calculate the drug or drug metabolite concentration of each sample in the unit ng/mL.

NOTE: If a result of Calc.? or Samp.? alarm is obtained, review the Reaction Monitor data for the sample and compare with the Reaction Monitor data for the highest calibrator. The most likely cause is a high concentration of the analyte in the sample, in which case the absorbance value for the sample will be less than that of the highest calibrator. Make an appropriate dilution of the sample using the 0 ng/mL calibrator and rerun the sample. A normal drug-free urine may be substituted for the 0 ng/mL calibrator if the urine and procedure have been validated by the laboratory. To ensure that the sample was not over-diluted, the diluted result, prior to multiplying by the dilution factor, must be at least half the analyte cutoff value. If the diluted result falls below half the analyte cutoff value, 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 to interpret results of Calc.? or Samp.? alarms, or when estimating concentration in preparation for GC-MS.

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

Preliminary positive results should be confirmed by another method.

For the semiquantitative applications cobas c systems automatically calculate the drug or metabolite concentration of each sample in the unit ng/mL. Results equal to or greater than the respective cutoff value are considered preliminary positive. Concentration values below the respective cutoff indicate a negative result.

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 and/or its metabolites in the sample. 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.

Serum/plasma

Criterion: No cross-over at initial values of samples of 150 ng/mL and 450 ng/mL (control levels).

Icterus:[21] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[21] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).

Lipemia (native lipaemic samples):[21] No significant interference up to an L index of 100. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

Immunoglobulins: No significant interference from immunoglobulins up to a concentration of 16 g/L (simulated by human immunoglobulin A), up to a concentration of 70 g/L (simulated by human immunoglobulin G) and up to a concentration of 10 g/L (simulated by human immunoglobulin M).

Albumin: No significant interference from human serum albumin up to a concentration of 70 g/L.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely lowered results.

Urine

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a methadone stock solution. Samples were tested on a Roche/Hitachi 917 analyzer and the following results were obtained:

Substance	Concentration tested	% Methadone recovery
Acetone	1 %	111
Ascorbic acid	1.5 %	104
Bilirubin	0.25 mg/mL	92
Creatinine	5 mg/mL	104
Ethanol	1 %	108
Glucose	2 %	108
Hemoglobin	7.5 g/L	112
Human albumin	0.5 %	109
Oxalic acid	2 mg/mL	104
Sodium chloride	0.5 M	100
Sodium chloride	1 M	98
Urea	6 %	107

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[22]

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Expected values

Qualitative assay

Results of this assay distinguish preliminary positive (≥ 300 ng/mL) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Semiquantitative assay

Results of this assay yield only approximate cumulative concentrations of the drug and its metabolites (see "Analytical specificity" section).

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Serum/plasma

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Qualitative precision - 300 ng/mL

Cutoff (300)	Number tested	Correct results	Confidence level
Serum -75 %	84	84	> 95 % negative reading
ACQ-L	84	84	> 95 % negative reading
Cutoff serum	84	n.a.*	n.a.*
ACQ-H	84	84	> 95 % negative reading
Serum +75 %	84	84	> 95 % negative reading

*n.a. = not applicable

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Urine

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Semiquantitative precision

Repeatability	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine -75 %	96.5	4.94	5.1
Urine -50 %	158	3.41	2.2
DAT1N	245	3.38	1.4
DATCN	242	6.16	2.6
Cutoff urine	281	3.84	1.4
DAT1P	390	4.99	1.3
DATCP	382	5.94	1.6
Urine +50 %	438	5.95	1.4
Urine +75 %	509	6.78	1.3
Urine +100 %	603	7.11	1.2
Intermediate precision	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine -75 %	89.1	9.08	10.2
Urine -50 %	158	7.49	4.7
DAT1N	240	7.55	3.2
DATCN	242	8.29	3.4
Cutoff urine	281	9.50	3.4
DAT1P	390	8.92	2.3
DATCP	382	10.9	2.9
Urine +50 %	438	11.5	2.6
Urine +75 %	509	17.9	3.5
Urine +100 %	621	16.2	2.6

Qualitative precision

Cutoff (300)	Number tested	Correct results	Confidence level
Urine -100 %	84	84	> 95 % negative reading
Urine -75 %	84	84	> 95 % negative reading
Urine -50 %	84	84	> 95 % negative reading
DAT1N	84	84	> 95 % negative reading
DATCN	84	84	> 95 % negative reading

Cutoff urine	84	n.a.*	n.a.*
DAT1P	84	84	> 95 % positive reading
DATCP	84	84	> 95 % positive reading
Urine +50 %	84	84	> 95 % positive reading
Urine +75 %	84	84	> 95 % positive reading
Urine +100 %	84	84	> 95 % positive reading

*n.a. = not applicable

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Accuracy

Serum/plasma

103 serum samples, screened negative for methadone on a **cobas c** 501 analyzer were evaluated with the Methadone II assay on a **cobas c** 503 analyzer. 100 % of these normal serum samples were negative with the Methadone II assay on a **cobas c** 503 analyzer. 51 serum samples screened positive for methadone relative to the 300 ng/mL cutoff on a **cobas c** 501 analyzer were evaluated with the Methadone II assay on a **cobas c** 503 analyzer. At the 300 ng/mL cutoff, 100 % of the samples were positive on both the **cobas c** 501 analyzer and the **cobas c** 503 analyzer.

Methadone II correlation (cutoff = 300 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	51	0
	-	0	103

52 serum samples, screened negative for methadone on a **cobas c** 501 analyzer were evaluated with the Methadone II assay on a **cobas c** 303 analyzer. 100 % of these normal serum samples were negative with the Methadone II assay on a **cobas c** 303 analyzer. 52 serum samples screened positive for methadone relative to the 300 ng/mL cutoff on a **cobas c** 501 analyzer were evaluated with the Methadone II assay on a **cobas c** 303 analyzer. At the 300 ng/mL cutoff, 100 % of the samples were positive on both the **cobas c** 501 analyzer and the **cobas c** 303 analyzer.

Methadone II correlation (cutoff = 300 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 303 analyzer	+	52	0
	-	0	52

Urine

45 urine samples, obtained from a clinical laboratory, where they screened negative in a drug test panel, were evaluated with the Methadone II assay. 100 % of these normal urines were negative relative to the 300 ng/mL cutoff. 44 urine samples obtained from a clinical laboratory, where they screened preliminary positive with a commercially available immunoassay, were subsequently confirmed by GC-MS and were evaluated with the Methadone II assay. 100 % of these samples were positive relative to the 300 ng/mL cutoff. In addition, 5 samples obtained from a clinical laboratory were found in a concentration of 75-100 % of the cutoff concentration and 6 samples obtained from a clinical laboratory were found in a concentration of 100-125 % of the cutoff concentration. The following results were obtained with the Methadone II assay on the **cobas c** 503 analyzer relative to the GC-MS values.

Methadone II clinical correlation (cutoff = 300 ng/mL)

		Negative samples	GC-MS values (ng/mL)		
			Near cutoff		Positive samples
			228-250	312-370	493-1703
cobas c 503 analyzer	+	0	0	6	44
	-	45	5	0	0

Additional clinical samples were evaluated with this assay on a **cobas c** 503 analyzer and on a **cobas c** 501 analyzer. 50 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Methadone II assay. 100 % of these normal urines were negative on both the **cobas c** 503 analyzer and the **cobas c** 501 analyzer. 50 urine samples, obtained from a clinical laboratory where they screened preliminary positive with a commercially available immunoassay were subsequently confirmed by GC-MS and evaluated with the Methadone II assay. 100 % of the samples were positive on both the **cobas c** 503 analyzer and the **cobas c** 501 analyzer.

Methadone II correlation (cutoff = 300 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	50	0
	-	0	50

Additional clinical samples were evaluated with this assay on a **cobas c** 303 analyzer and on a **cobas c** 501 analyzer. 111 urine samples screened negative for methadone on a **cobas c** 501 analyzer were evaluated with the Methadone II assay on a **cobas c** 303 analyzer. 100 % of these normal urines were negative on both the **cobas c** 303 analyzer and the **cobas c** 501 analyzer. 50 urine samples, obtained from a clinical laboratory where they screened preliminary positive with a commercially available immunoassay were subsequently confirmed by GC-MS and evaluated with the Methadone II assay on a **cobas c** 303 analyzer. 100 % of the samples were positive on both the **cobas c** 501 analyzer and the **cobas c** 303 analyzer.

Methadone II correlation (cutoff = 300 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 303 analyzer	+	50	0
	-	0	111

Analytical specificity

Serum/plasma

The specificity of this 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 a 300 ng/mL assay cutoff. Caution should be taken when interpreting results of patient samples containing structurally related compounds having greater than 0.5 % cross-reactivity. The following results were obtained on a **cobas c** 501 analyzer.

Compound	**ng/mL Equivalent to 300 ng/mL methadone**	**Approximate % cross-reactivity**
Chlorpromazine	70752	0.42
Metabolite Lu AA34443	6341	4.73
Methadone	308	97.3
Vortioxetine	8344	3.60

Urine

The specificity of this 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 a 300 ng/mL assay cutoff. Caution should be taken when interpreting results of patient samples containing structurally related compounds having greater than 0.5 % cross-reactivity. The following results were obtained on Roche/Hitachi 917 and **cobas c** analyzers.

Compound[b)]	**ng/mL Equivalent to 300 ng/mL methadone**	**Approximate % cross-reactivity**

Hydroxymethadone	3289	9.1
Vortioxetine	7339	4.1
Lu AA34443	2622	11
Cyamemazine	8477	3.5
Methotrimeprazine (Levomepromazine)	8939	3.4
Chlorpromazine	26071	1.2
Thiothixene	39267	0.8
Clomipramine	135747	0.2
Promazine	142857	0.2
Thioridazine	146341	0.2
Chlorprothixene	186335	0.2
l-α-methadol	220588	0.1
Promethazine	288462	0.1
l-α-acetylmethadol (LAAM)	370370	0.1
Trimipramine	422535	0.1

b) Indented compounds are metabolites of the preceding drug.

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

Amitriptyline	EMDP (2-ethyl-5-methyl-3,3-diphenylpyrroline)
Benzphetamine	
Carbamazepine	Fluoxetine
Chlorpheniramine	Imipramine
Cyclobenzaprine	Maprotiline
Cyproheptadine	Meperidine
Desipramine	Mianserin
Dextromethorphan	Nordoxepin
Diphenhydramine	Nortriptyline
Disopyramide	Orphenadrine
Doxepin	Perphenazine
Doxylamine	*d*-Propoxyphene
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	Protriptyline
	d,l-Verapamil

The cross-reactivity for disopyramide at a concentration of 1 mg/mL was tested with the Methadone II assay. The result obtained was < 0.01 %. Specimens from Seroquel (quetiapine fumarate) users have screened positive for methadone.

Drug interference

Serum/plasma

Interfering substances were added to serum containing methadone at -50 % and +50 % of the cutoff level at the concentration listed below. Samples were tested and the following results were obtained on a **cobas c** 501 analyzer.

Compound	**Comp. conc. mg/L**	**Neg. level**	**Pos. level**
Acetaminophen	200	neg	pos
Acetylcysteine	1660	neg	pos
Acetylsalicylic acid	1000	neg	pos
Amitriptyline	1.00	neg	pos
Ampicillin-Na	1000	neg	pos
Ascorbic acid	300	neg	pos
Caffeine	59.8	neg	pos
Cefoxitin	2500	neg	pos
Cyclosporine	5.00	neg	pos
d-Amphetamine	1.36	neg	pos
Diazepam	5.13	neg	pos
Doxycycline	50.0	neg	pos
d-Pseudoephedrine	9.98	neg	pos
Erythromycin	59.9	neg	pos
Fenoprofen	195	neg	pos
Furosemide	59.9	neg	pos
Gentisic acid	18.0	neg	pos
Heparin	5000 U/L	neg	pos
Hydrochlorothiazide	6.02	neg	pos
l-Amphetamine	1.00	neg	pos
Ibuprofen	500	neg	pos
Imipramine	0.70	neg	pos
Ketamine	10.0	neg	pos
Levodopa	20.0	neg	pos
Lidocaine	12.0	neg	pos
Methyldopa + 1.5 H_2O	20.0	neg	pos
Metronidazole	200	neg	pos
Morphine	0.50	neg	pos
Naproxen	499	neg	pos
Phenylbutazone	400	neg	pos
Procaine	39.9	neg	pos
Promethazine	1.20	neg	pos
Quinidine	12.0	neg	pos
Quinine	48.0	neg	pos
Rifampicin	60.0	neg	pos
Tetracycline	15.1	neg	pos
Theophylline	100	neg	pos
Trifluoperazine	1.00	neg	pos

Urine

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, and no results were greater than the assay cutoff (300 ng/mL).

Acetaminophen	LSD
Acetylsalicylic acid	MDA
Aminopyrine	MDMA
Amobarbital	Melanin
d-Amphetamine	*d*-Methamphetamine
l-Amphetamine	*l*-Methamphetamine
Ampicillin	Methaqualone
Ascorbic acid	Methylphenidate
Aspartame	Methyprylon
Atropine	Morphine sulfate
Benzocaine	Naloxone
Benzoylecgonine (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	Phencyclidine
Dopamine	*β*-Phenethylamine
Ecgonine	Phenobarbital
Ecgonine methyl ester	Phenothiazine
d-Ephedrine	Phentermine
d,l-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
Lidocaine	

The cross-reactivity for tramadol, at a concentration of 102465 ng/mL, is 0.3 %.
The cross-reactivity for ofloxacin, at a concentration of 220000 ng/mL, is 0.1 %.

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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 for reconstitution
GTIN	Global Trade Item Number

MDN2

ONLINE DAT Methadone II

Rx only

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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