# AMPII

cobas®

**ONLINE DAT Amphetamines II**

**Drug abuse testing**

**Order information**

| REF | CONTENT | | Analyzers on which **cobas c** pack can be used |
|---|---|---|---|
| **04512936** 190 | ONLINE DAT Amphetamines II (200 tests) | System-ID 07 6835 9 | COBAS INTEGRA 400 plus<br>COBAS INTEGRA 800 |
| **03304671** 190 | Preciset DAT Plus I CAL 1-6 (6 × 5 mL) | | |
| **03304680** 190 | Preciset DAT Plus II 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) | | |
| **03312968** 190 | Control Set DAT II (for 300 ng/mL assay)<br>PreciPos DAT Set II (2 × 10 mL)<br>PreciNeg DAT Set II (2 × 10 mL) | | |
| **03312950** 190 | Control Set DAT I (for 500 ng/mL assay<br>PreciPos DAT Set I (2 × 10 mL)<br>PreciNeg DAT Set I (2 × 10 mL) | | |
| **04500873** 190 | Control Set DAT Clinical (for 500 ng/mL assay<br>PreciPos DAT Clinical (2 × 10 mL)<br>PreciNeg DAT Clinical (2 × 10 mL) | | |
| **03312976** 190 | Control Set DAT III (for 1000 ng/mL assay)<br>PreciPos DAT Set III (2 × 10 mL)<br>PreciNeg DAT Set III (2 × 10 mL) | | |

**English**

**System information**

Test AM3S2, test-ID 0-358 for semiquantitative assay, 300 ng/mL

Test AM5S2, test-ID 0-359 for semiquantitative assay, 500 ng/mL

Test AM1S2, test-ID 0-360 for semiquantitative assay, 1000 ng/mL

Test AM3Q2, test-ID 0-330 for qualitative assay, 300 ng/mL

Test AM5Q2, test-ID 0-340 for qualitative assay, 500 ng/mL

Test AM1Q2, test-ID 0-350 for qualitative assay, 1000 ng/mL

Test AM5QC, test-ID 0-341 for qualitative assay, 500 ng/mL using C.f.a.s. DAT Qualitative Plus Clinical

**Intended use**

Amphetamines II (AMPII) is an in vitro diagnostic test for the qualitative and semiquantitative detection of amphetamines and methamphetamines in human urine on COBAS INTEGRA systems at cutoff concentrations of 300 ng/mL, 500 ng/mL and 1000 ng/mL when calibrated with *d*-methamphetamine.

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).

**Amphetamines 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 judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.**

**Summary**

The amphetamines are known as the sympathomimetic amines as they mimic the effects of stimulation of the sympathetic nervous system. These small molecules, based on ß-phenylethylamine, structurally resemble the bodies own catecholamines. A wide variety have been created via substitutions anywhere on the structure. The amphetamines are potent central nervous stimulants. As such they can increase wakefulness, physical activity, and decrease appetite. The amphetamines have some limited indications and approval for use in ADHD, narcolepsy, and obesity. However, because these CNS stimulants convey a sense of self-confidence, well being, and euphoria, they are highly addictive, widely abused, and consequently controlled substances.[2] Abuse can lead to medical, psychological, and social consequences. Adverse health effects include memory loss, aggression, psychotic behavior, heart damage, malnutrition, and severe dental problems.[3] Amphetamine may be self-administered either orally or by intravenous injection in amounts of up to 2000 mg daily by tolerant addicts. It is a metabolite of a number of other drugs including methamphetamine. Normally about 30 % is excreted unchanged in the 24 hour urine, but this may change to as much as 74 % in acid urine and may decrease to 1 % in alkaline urine.[4]

Amphetamines II is calibrated with *d*-methamphetamine and therefore the sensitivity towards amphetamines is different than *d*-methamphetamine, as indicated in the “Analytical specificity” section.

**Test principle**

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[5,6] as measured by changes in light transmission. In the absence of amphetamines and/or methamphetamines, soluble drug conjugates bind to antibody-bound microparticles, causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of amphetamines and/or methamphetamines, the absorbance increases.

When a urine sample contains amphetamines and/or methamphetamines, this drug competes with the drug derivative conjugate for microparticle-bound antibody. Antibody bound to amphetamine and/or methamphetamine is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of amphetamines and/or methamphetamines diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Amphetamines and/or methamphetamines content is determined relative to the value obtained for a known cutoff concentration of *d*-methamphetamine.

**Reagents - working solutions**

**R1** Conjugated amphetamine and methamphetamine derivatives; buffer; bovine serum albumin; 0.09 % sodium azide

**SR** Microparticles attached to amphetamine and methamphetamine antibodies (mouse monoclonal); buffer; bovine serum albumin; 0.09 % sodium azide

R1 is in position A 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.[7]

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*.[8]

**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 | 659 nm | 659 nm | |
| Test range | | | |
| *AM3S2* | 0-2000 ng/mL | 0-2000 | *AM3Q2* |
| *AM5S2, AM1S2* | 0-5000 ng/mL | 0-5000 | *AM5Q2, AM5QC, AM1Q2* |
| with postdilution | | | |
| *AM3S2* | 0-20000 ng/mL | | |
| *AM5S2, AM1S2* | 0-50000 ng/mL | | |
| Postdilution factor | 10 recommended[a)] | No | |
| Calc. first/last | 51/70 | 51/70 | |
| Unit | ng/mL | | |

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

**Pipetting parameters**

| *AM3S2, AM3Q2* | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 60 µL | 20 µL |
| Sample | 10 µL | 5 µL |
| SR | 47 µL | 5 µL |
| Total volume | 147 µL | |

| *AM5S2, AM5Q2, AM5QC* | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 60 µL | 20 µL |
| Sample | 7.5 µL | 5 µL |
| SR | 47 µL | 5 µL |
| Total volume | 144.5 µL | |

| *AM1S2, AM1Q2* | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 60 µL | 20 µL |
| Sample | 5 µL | 5 µL |
| SR | 47 µL | 5 µL |
| Total volume | 142 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 | 659 nm | 659 nm |
| Test range | | |
| *AM3S2* | 0-2000 ng/mL | 0-2000 |
| *AM5S2, AM1S2* | 0-5000 ng/mL | |
| with postdilution | | |
| *AM3S2* | 0-20000 ng/mL | |
| *AM5S2, AM1S2* | 0-50000 ng/mL | |
| Postdilution factor | 10 recommended[b)] | No |
| Calc. first/last | 46/86 | 46/86 |
| Unit | ng/mL | |

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

**Pipetting parameters**

| *AM3S2, AM3Q2* | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 60 µL | 20 µL |
| Sample | 10 µL | 5 µL |
| SR | 47 µL | 3 µL |
| Total volume | 145 µL | |

| *AM5S2, AM5Q2, AM5QC* | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 60 µL | 20 µL |
| Sample | 7.5 µL | 5 µL |
| SR | 47 µL | 3 µL |
| Total volume | 142.5 µL | |

| *AM1S2, AM1Q2* | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 60 µL | 20 µL |
| Sample | 5 µL | 5 µL |
| SR | 47 µL | 3 µL |
| Total volume | 140 µL | |

**Calibration**

Calibrators

*Semiquantitative applications*

*AM3S2, 0-358* Preciset DAT Plus II calibrators, CAL 1-6
0, 150, 300, 600, 1000, 2000 ng/mL
*d*-methamphetamine
(300 cutoff, DATS8, system-ID 07 6796 4

*AM5S2, 0-359; AM1S2, 0-360* Preciset DAT Plus I calibrators, CAL 1-6
0, 250, 500, 1000, 3000, 5000 ng/mL
*d*-methamphetamine
(500 and 1000 cutoffs, DATS2,
system-ID 07 6764 6)

*Qualitative applications*

*AM3Q2, 0-330* Preciset DAT Plus II calibrators, CAL 1
0 ng/mL *or* deionized water
*and*
Preciset DAT Plus II calibrators, CAL 3
300 ng/mL
(300 cutoff, DATQ3, system-ID 07 6770 0)

For qualitative applications, the cutoff of 300 ng/mL is assigned a value of 1000.

*AM5Q2, 0-340* (Preciset DAT Plus I calibrators, CAL 1)
0 ng/mL *or* deionized water
*and*
C.f.a.s. DAT Qualitative Plus
500 ng/mL
(500 cutoff, DATQ1, system-ID 07 6744 1)

For qualitative applications, the cutoff of 500 ng/mL is assigned a value of 1000.

*AM5QC, 0-341* Preciset DAT Plus I or II calibrators, CAL 1
0 ng/mL *or* deionized water
*and*
C.f.a.s. DAT Qualitative Plus Clinical
500 ng/mL
(500 cutoff, DATQ5, system-ID 07 6880 4)

For qualitative applications, the cutoff of 500 ng/mL is assigned a value of 1000.

*AM1Q2, 0-350* Preciset DAT Plus I calibrators, CAL 1
0 ng/mL *or* deionized water
*and*
Preciset DAT Plus I calibrators, CAL 4
1000 ng/mL
(1000 cutoff, DATQ2, system-ID 07 6768 9)

For qualitative applications, the cutoff of 1000 mg/dL is assigned a value of 1000.

Calibration mode

*Semiquantitative applications*

COBAS INTEGRA 400 plus analyzer:
Logit/log 5 (AM1S2, 0-360)
Logit/log 4 (AM3S2, 0-358; AM5S2, 0-359)

COBAS INTEGRA 800 analyzer:
Logit/log 4

*Qualitative applications*

Calibration replicate

Linear regression

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

*300 ng/mL cutoff*

Control Set DAT II

PreciPos DAT Set II
(DAT2P, system-ID 07 6771 9)

PreciNeg DAT Set II
(DAT2N, system-ID 07 6772 7)

*500 ng/mL cutoff*

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)

*1000 ng/mL cutoff*

Control Set DAT III

PreciPos DAT Set III
(DAT3P, system-ID 07 6773 5)

PreciNeg DAT Set III
(DAT3N, system-ID 07 6774 3)

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, II, III, 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*
*AM3S2 (300 ng/mL cutoff)*

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| No flag | Negative | < 300 ng/mL |

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| <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.

*Semiquantitative result reporting*
*AM5S2 (500 ng/mL cutoff)*

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| No flag | Negative | < 500 ng/mL |
| <TEST RNG | Negative | < 0 ng/mL |
| >TEST RNG | Positive | > 5000 ng/mL |
| POS 500 | Positive | ≥ 500 ng/mL |

Value ranges listed above are based on a cutoff value of 500 ng/mL.

*Semiquantitative result reporting*
*AM1S2 (1000 ng/mL cutoff)*

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| No flag | Negative | < 1000 ng/mL |
| <TEST RNG | Negative | < 0 ng/mL |
| >TEST RNG | Positive | > 5000 ng/mL |
| POS 1000 | Positive | ≥ 1000 ng/mL |

Value ranges listed above are based on a cutoff value of 1000 ng/mL.

*Qualitative result reporting*
*AM3Q2 (300 ng/mL cutoff)*

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| No flag | Negative | < 1000 |
| <TEST RNG | Negative | < 0 |
| >TEST RNG | Positive | > 2000 |
| POS 1000 | Positive | ≥ 1000 |

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

*Qualitative result reporting*
*AM5Q2, AM5QC (500 ng/mL cutoff)*

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| No flag | Negative | < 1000 |
| <TEST RNG | Negative | < 0 |
| >TEST RNG | Positive | > 2000 |
| POS 1000 | Positive | ≥ 1000 |

Value ranges above are based on assigning the cutoff of 500 ng/mL a value of 1000.

*Qualitative result reporting*
*AM1Q2 (1000 ng/mL cutoff)*

| Flag | COBAS INTEGRA | Value range |
|---|---|---|
| No flag | Negative | < 1000 |
| <TEST RNG | Negative | < 0 |
| >TEST RNG | Positive | > 2000 |
| POS 1000 | Positive | ≥ 1000 |

Value ranges above are based on assigning the cutoff of 1000 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 amphetamines and/or methamphetamines 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.

**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.**

**Expected values**

No drug should be present in individuals that have not ingested amphetamines and/or methamphetamines.

**Specific performance data**

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

**Precision**

A *d*-methamphetamine (MAMP) solution (1 mg/mL) was added to 9 samples obtained from a human urine sample pool to achieve concentrations at approximately –100 %, –75 %, –50 %, –25 %, ±0 %, +25 %, +50 %, +75 %, and +100 % of the cutoff value. These samples were tested for precision in qualitative and semiquantitative modes. Following a CLSI (EP5-A2) precision protocol, samples were tested in 2 replicates per run, 2 runs per day for 21 days, total n = 84.

*Qualitative - 300 ng/mL Cutoff*

| Drug | Concentration of Sample | Number of Determinations | Results # Neg / # Pos |
|---|---|---|---|
| MAMP | zero drug | 84 | 84 Neg / 0 Pos |
| MAMP | –75 % | 84 | 84 Neg / 0 Pos |
| MAMP | –50 % | 84 | 84 Neg / 0 Pos |
| MAMP | –25 % | 84 | 83 Neg / 1 Pos |
| MAMP | cutoff | 84 | 27 Neg / 57 Pos |
| MAMP | +25 % | 84 | 0 Neg / 84 Pos |
| MAMP | +50 % | 84 | 0 Neg / 84 Pos |
| MAMP | +75 % | 84 | 0 Neg / 84 Pos |
| MAMP | +100 % | 84 | 0 Neg / 84 Pos |

*Qualitative - 500 ng/mL Cutoff*

| Drug | Concentration of Sample | Number of Determinations | Results # Neg / # Pos |
|---|---|---|---|
| MAMP | zero drug | 84 | 84 Neg / 0 Pos |
| MAMP | –75 % | 84 | 84 Neg / 0 Pos |
| MAMP | –50 % | 84 | 84 Neg / 0 Pos |
| MAMP | –25 % | 84 | 84 Neg / 0 Pos |
| MAMP | cutoff | 84 | 28 Neg / 56 Pos |
| MAMP | +25 % | 84 | 0 Neg / 84 Pos |
| MAMP | +50 % | 84 | 0 Neg / 84 Pos |
| MAMP | +75 % | 84 | 0 Neg / 84 Pos |
| MAMP | +100 % | 84 | 0 Neg / 84 Pos |

*Qualitative - 1000 ng/mL Cutoff*

| Drug | Concentration of Sample | Number of Determinations | Results # Neg / # Pos |
|---|---|---|---|
| MAMP | zero drug | 84 | 84 Neg / 0 Pos |
| MAMP | –75 % | 84 | 84 Neg / 0 Pos |
| MAMP | –50 % | 84 | 84 Neg / 0 Pos |
| MAMP | –25 % | 84 | 84 Neg / 0 Pos |
| MAMP | cutoff | 84 | 13 Neg / 71 Pos |
| MAMP | +25 % | 84 | 0 Neg / 84 Pos |
| MAMP | +50 % | 84 | 0 Neg / 84 Pos |
| MAMP | +75 % | 84 | 0 Neg / 84 Pos |
| MAMP | +100 % | 84 | 0 Neg / 84 Pos |

*Semiquantitative - 300 ng/mL Cutoff*

| Drug | Sample Conc. | Results # Neg / # Pos | Repeatability | | Intermediate Precision | |
|---|---|---|---|---|---|---|
| | | | SD ng/mL | CV % | SD ng/mL | CV % |
| MAMP | zero drug | 84 / 0 | 9.4 | 95.4 | 12.2 | 123.8 |
| MAMP | –75 % | 84 / 0 | 12.9 | 15.5 | 15.7 | 18.8 |
| MAMP | –50 % | 84 / 0 | 14.3 | 9.0 | 17.7 | 11.2 |
| MAMP | –25 % | 84 / 0 | 13.2 | 5.4 | 19.8 | 8.2 |
| MAMP | cutoff | 34 / 50 | 23.2 | 7.6 | 23.5 | 7.7 |
| MAMP | +25 % | 0 / 84 | 20.9 | 5.2 | 24.4 | 6.1 |
| MAMP | +50 % | 0 / 84 | 22.5 | 5.0 | 29.5 | 6.6 |
| MAMP | +75 % | 0 / 84 | 27.4 | 5.0 | 36.5 | 6.6 |
| MAMP | +100 % | 0 / 84 | 35.7 | 5.7 | 40.8 | 6.5 |

*Semiquantitative - 500 ng/mL Cutoff*

| Drug | Sample Conc. | Results # Neg / # Pos | Repeatability | | Intermediate Precision | |
|---|---|---|---|---|---|---|
| | | | SD ng/mL | CV % | SD ng/mL | CV % |
| MAMP | zero drug | 84 / 0 | 9.6 | 117.3 | 11.0 | 134.1 |
| MAMP | –75 % | 84 / 0 | 14.6 | 10.5 | 20.6 | 14.9 |
| MAMP | –50 % | 84 / 0 | 20.0 | 7.9 | 21.9 | 8.6 |
| MAMP | –25 % | 84 / 0 | 24.1 | 6.2 | 27.2 | 7.0 |
| MAMP | cutoff | 39 / 45 | 28.2 | 5.6 | 39.4 | 7.8 |
| MAMP | +25 % | 0 / 84 | 31.5 | 4.9 | 48.5 | 7.5 |
| MAMP | +50 % | 0 / 84 | 34.5 | 4.8 | 49.5 | 6.8 |
| MAMP | +75 % | 0 / 84 | 41.6 | 5.0 | 62.3 | 7.5 |
| MAMP | +100 % | 0 / 84 | 46.7 | 4.6 | 72.4 | 7.1 |

*Semiquantitative - 1000 ng/mL Cutoff*

| Drug | Sample Conc. | Results # Neg / # Pos | Repeatability | | Intermediate Precision | |
|---|---|---|---|---|---|---|
| | | | SD ng/mL | CV % | SD ng/mL | CV % |
| MAMP | zero drug | 84 / 0 | 20.2 | 97.2 | 24.9 | 119.5 |
| MAMP | –75 % | 84 / 0 | 25.3 | 9.3 | 30.7 | 11.2 |
| MAMP | –50 % | 84 / 0 | 33.3 | 6.4 | 39.4 | 7.5 |
| MAMP | –25 % | 84 / 0 | 34.3 | 4.6 | 52.1 | 7.0 |
| MAMP | cutoff | 25 / 59 | 56.9 | 5.5 | 71.3 | 6.9 |
| MAMP | +25 % | 0 / 84 | 56.6 | 4.2 | 79.3 | 5.9 |
| MAMP | +50 % | 0 / 84 | 63.6 | 4.0 | 81.3 | 5.2 |
| MAMP | +75 % | 0 / 84 | 60.3 | 3.5 | 101.6 | 5.9 |
| MAMP | +100 % | 0 / 84 | 108.1 | 5.1 | 126.6 | 6.0 |

The same precision experiment was repeated utilizing *d*-amphetamine (AMP) as the target analyte instead of *d*-methamphetamine. The following tables show the results obtained on a COBAS INTEGRA 800 analyzer.

*Qualitative - 300 ng/mL Cutoff*

| Drug | Concentration of Sample | Number of Determinations | Results # Neg / # Pos |
|---|---|---|---|
| AMP | zero drug | 84 | 84 Neg / 0 Pos |
| AMP | –75 % | 84 | 84 Neg / 0 Pos |
| AMP | –50 % | 84 | 84 Neg / 0 Pos |
| AMP | –25 % | 84 | 83 Neg / 1 Pos |
| AMP | cutoff | 84 | 2 Neg / 82 Pos |
| AMP | +25 % | 84 | 0 Neg / 84 Pos |
| AMP | +50 % | 84 | 0 Neg / 84 Pos |
| AMP | +75 % | 84 | 0 Neg / 84 Pos |
| AMP | +100 % | 84 | 0 Neg / 84 Pos |

*Qualitative - 500 ng/mL Cutoff*

| Drug | Concentration of Sample | Number of Determinations | Results # Neg / # Pos |
|---|---|---|---|
| AMP | zero drug | 84 | 84 Neg / 0 Pos |
| AMP | –75 % | 84 | 84 Neg / 0 Pos |
| AMP | –50 % | 84 | 84 Neg / 0 Pos |
| AMP | –25 % | 84 | 82 Neg / 2 Pos |
| AMP | cutoff | 84 | 6 Neg / 78 Pos |
| AMP | +25 % | 84 | 0 Neg / 84 Pos |
| AMP | +50 % | 84 | 0 Neg / 84 Pos |
| AMP | +75 % | 84 | 0 Neg / 84 Pos |
| AMP | +100 % | 84 | 0 Neg / 84 Pos |

*Qualitative - 1000 ng/mL Cutoff*

| Drug | Concentration of Sample | Number of Determinations | Results # Neg / # Pos |
|---|---|---|---|
| AMP | zero drug | 84 | 84 Neg / 0 Pos |
| AMP | –75 % | 84 | 84 Neg / 0 Pos |
| AMP | –50 % | 84 | 84 Neg / 0 Pos |
| AMP | –25 % | 84 | 82 Neg / 2 Pos |
| AMP | cutoff | 84 | 7 Neg / 77 Pos |
| AMP | +25 % | 84 | 1 Neg / 83 Pos |
| AMP | +50 % | 84 | 0 Neg / 84 Pos |
| AMP | +75 % | 84 | 0 Neg / 84 Pos |
| AMP | +100 % | 84 | 0 Neg / 84 Pos |

*Semiquantitative - 300 ng/mL Cutoff*

| Drug | Sample Conc. | Results # Neg / # Pos | Repeatability | | Intermediate Precision | |
|---|---|---|---|---|---|---|
| | | | SD ng/mL | CV % | SD ng/mL | CV % |
| AMP | zero drug | 84 / 0 | 8.6 | 110.0 | 10.6 | 135.7 |
| AMP | –75 % | 84 / 0 | 10.4 | 16.8 | 13.6 | 21.9 |
| AMP | –50 % | 84 / 0 | 14.6 | 10.0 | 19.9 | 13.6 |
| AMP | –25 % | 84 / 0 | 15.7 | 6.6 | 20.6 | 8.7 |
| AMP | cutoff | 4 / 80 | 25.8 | 7.4 | 28.5 | 8.2 |
| AMP | +25 % | 0 / 84 | 30.7 | 7.1 | 32.2 | 7.4 |
| AMP | +50 % | 0 / 84 | 31.1 | 6.1 | 37.6 | 7.3 |
| AMP | +75 % | 0 / 84 | 29.1 | 4.9 | 41.2 | 7.0 |
| AMP | +100 % | 0 / 84 | 36.2 | 5.3 | 44.6 | 6.5 |

*Semiquantitative - 500 ng/mL Cutoff*

| Drug | Sample Conc. | Results # Neg / # Pos | Repeatability | | Intermediate Precision | |
|---|---|---|---|---|---|---|
| | | | SD ng/mL | CV % | SD ng/mL | CV % |
| AMP | zero drug | 84 / 0 | 12.6 | 117.0 | 13.2 | 122.5 |
| AMP | –75 % | 84 / 0 | 21.4 | 16.9 | 25.2 | 20.0 |
| AMP | –50 % | 84 / 0 | 18.2 | 6.9 | 25.6 | 9.8 |
| AMP | –25 % | 83 / 1 | 25.6 | 5.7 | 29.7 | 6.6 |
| AMP | cutoff | 0 / 84 | 29.1 | 4.7 | 38.3 | 6.2 |
| AMP | +25 % | 0 / 84 | 42.0 | 5.5 | 51.7 | 6.8 |
| AMP | +50 % | 0 / 84 | 40.3 | 4.6 | 49.9 | 5.7 |
| AMP | +75 % | 0 / 84 | 58.7 | 5.9 | 59.7 | 6.0 |
| AMP | +100 % | 0 / 84 | 79.0 | 7.0 | 86.3 | 7.6 |

*Semiquantitative - 1000 ng/mL Cutoff*

| Drug | Sample Conc. | Results # Neg / # Pos | Repeatability | | Intermediate Precision | |
|---|---|---|---|---|---|---|
| | | | SD ng/mL | CV % | SD ng/mL | CV % |
| AMP | zero drug | 84 / 0 | 15.9 | 111.1 | 19.0 | 132.6 |
| AMP | –75 % | 84 / 0 | 21.9 | 9.2 | 29.8 | 12.5 |
| AMP | –50 % | 84 / 0 | 30.3 | 5.4 | 40.9 | 7.3 |
| AMP | –25 % | 84 / 0 | 44.3 | 5.2 | 55.0 | 6.4 |
| AMP | cutoff | 6 / 78 | 53.4 | 4.8 | 84.3 | 7.6 |
| AMP | +25 % | 0 / 84 | 72.5 | 5.6 | 87.5 | 6.7 |
| AMP | +50 % | 0 / 84 | 65.7 | 4.4 | 96.6 | 6.5 |
| AMP | +75 % | 0 / 84 | 74.3 | 4.5 | 105.2 | 6.4 |
| AMP | +100 % | 0 / 84 | 106.7 | 5.9 | 131.4 | 7.3 |

**Accuracy**

The accuracy of this assay was determined against *d*-methamphetamine or *d*-amphetamine GC/MS results. The evaluated cutoff concentrations for the GC/MS testing were the same as the assay screening cutoffs. For both *d*-methamphetamine and *d*-amphetamine, 36 urine samples, obtained from a clinical laboratory were confirmed to be negative by GC/MS and were evaluated with Amphetamines II. 100 % of these normal urines were negative with both the semiquantitative and qualitative assay relative to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL cutoffs. 4 additional unaltered samples, containing *d*-methamphetamine between 85-204 ng/mL (GC/MS confirmed), were tested with the 500 ng/mL cutoff and gave negative results.

36 samples obtained from a clinical laboratory, where they screened preliminary positive with a commercially available enzyme immunoassay and were subsequently confirmed positive by GC/MS to contain *d*-methamphetamine or *d*-amphetamine, were evaluated with Amphetamines II. 100 % of these samples were positive relative to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL cutoffs.

In addition, several unaltered near cutoff samples were run for each cutoff. These samples fell in the near cutoff negative range (between –50 % and cutoff) and the near cutoff positive range (between cutoff and +50 %). For each cutoff, 4 negative near cutoff samples and 4 positive near cutoff samples were assayed.

Data from the accuracy studies described above were combined with data generated from the unaltered near cutoff urine samples. The following results were obtained with the Amphetamines II assay on the COBAS INTEGRA 800 analyzer relative to the GC/MS values for both *d*-methamphetamine and *d*-amphetamine.

**Amphetamines II Qualitative Assay Results (MAMP)**

| Roche ONLINE DAT AMPII assay | Low Neg | Near Cutoff Negative by GC/MS (between –50 % and cutoff) | Near Cutoff Positive by GC/MS (between cutoff and +50 %) | High Positive by GC/MS (greater than +50 %) | Percent Agreement with GC/MS (MAMP) |
|---|---|---|---|---|---|
| *300 ng/mL Cutoff* | | | | | |
| Positive | 0 | 4 | 4 | 36 | 100 % |
| Negative | 36 | 0 | 0 | 0 | 90 % |
| *500 ng/mL Cutoff* | | | | | |
| Positive | 0 | 4 | 4 | 36 | 100 % |
| Negative | 40 | 0 | 0 | 0 | 91 % |
| *1000 ng/mL Cutoff* | | | | | |
| Positive | 0 | 4 | 4 | 36 | 100 % |
| Negative | 36 | 0 | 0 | 0 | 90 % |

**Amphetamines II Semiqualitative Assay Results (MAMP)**

| Roche ONLINE DAT AMPII assay | Low Neg | Near Cutoff Negative by GC/MS (between –50 % and cutoff) | Near Cutoff Positive by GC/MS (between cutoff and +50 %) | High Positive by GC/MS (greater than +50 %) | Percent Agreement with GC/MS (MAMP) |
|---|---|---|---|---|---|
| *300 ng/mL Cutoff* | | | | | |
| Positive | 0 | 4 | 4 | 36 | 100 % |
| Negative | 36 | 0 | 0 | 0 | 90 % |
| *500 ng/mL Cutoff* | | | | | |
| Positive | 0 | 4 | 4 | 36 | 100 % |
| Negative | 40 | 0 | 0 | 0 | 91 % |
| *1000 ng/mL Cutoff* | | | | | |
| Positive | 0 | 4 | 4 | 36 | 100 % |
| Negative | 36 | 0 | 0 | 0 | 90 % |

Accuracy samples were categorized based upon the *d*-methamphetamine GC/MS concentration only. The table below identifies those samples with a *d*-methamphetamine concentration below the cutoff, in which the observed result on a COBAS INTEGRA 800 analyzer was positive. The expected results column identifies the result expected with the Amphetamines II assay based upon the *d*-methamphetamine (MAMP) value relative to the cutoff.

**GC/MS Summary of Discrepant Results (MAMP)**

| Cutoff Value (ng/mL) | Roche ONLINE DAT AMPII OBSERVED Result | Roche ONLINE DAT AMPII EXPECTED Result | GC/MS (ng/mL) | Drug / Metabolite |
|---|---|---|---|---|
| 300 (SQ, Q) | Positive | Positive | 173<br>181 | MAMP<br>AMP |
| 300 (SQ, Q) | Positive | Positive | 278<br>101 | MAMP<br>AMP |
| 300 (SQ, Q) | Positive | Positive | 171<br>220 | MAMP<br>AMP |
| 300 (SQ, Q) | Positive | Positive | 291<br>145 | MAMP<br>AMP |
| 500 (SQ, Q) | Positive | Positive | 488<br>466 | MAMP<br>AMP |
| 500 (SQ, Q) | Positive | Negative | 325<br>171 | MAMP<br>AMP |
| 500 (SQ, Q) | Positive | Negative | 291<br>145 | MAMP<br>AMP |
| 500 (SQ, Q) | Positive | Positive | 472<br>650 | MAMP<br>AMP |
| 1000 (SQ, Q) | Positive | Positive | 706<br>443 | MAMP<br>AMP |
| 1000 (SQ, Q) | Positive | Positive | 540<br>693 | MAMP<br>AMP |
| 1000 (SQ, Q) | Positive | Positive | 769<br>395 | MAMP<br>AMP |
| 1000 (SQ, Q) | Positive | Positive | 572<br>432 | MAMP<br>AMP |

**Amphetamines II Qualitative Assay Results (AMP)**

| Roche ONLINE DAT AMPII assay | Low Neg | Near Cutoff Negative by GC/MS (between –50 % and cutoff) | Near Cutoff Positive by GC/MS (between cutoff and +50 %) | High Positive by GC/MS (greater than +50 %) | Percent Agreement with GC/MS (AMP) |
|---|---|---|---|---|---|
| *300 ng/mL Cutoff* | | | | | |
| Positive | 0 | 3 | 4 | 36 | 100 % |
| Negative | 36 | 1 | 0 | 0 | 92.5 % |
| *500 ng/mL Cutoff* | | | | | |
| Positive | 0 | 3 | 4 | 36 | 100 % |
| Negative | 36 | 1 | 0 | 0 | 92.5 % |
| *1000 ng/mL Cutoff* | | | | | |
| Positive | 0 | 3 | 4 | 36 | 100 % |
| Negative | 36 | 1 | 0 | 0 | 97.5 % |

**Amphetamines II Semiqualitative Assay Results (AMP)**

| Roche ONLINE DAT AMPII assay | Low Neg | Near Cutoff Negative by GC/MS (between –50 % and cutoff) | Near Cutoff Positive by GC/MS (between cutoff and +50 %) | High Positive by GC/MS (greater than +50 %) | Percent Agreement with GC/MS (AMP) |
|---|---|---|---|---|---|
| *300 ng/mL Cutoff* | | | | | |
| Positive | 0 | 3 | 4 | 36 | 100 % |
| Negative | 36 | 1 | 0 | 0 | 92.5 % |
| *500 ng/mL Cutoff* | | | | | |
| Positive | 0 | 3 | 4 | 36 | 100 % |
| Negative | 36 | 1 | 0 | 0 | 92.5 % |
| *1000 ng/mL Cutoff* | | | | | |
| Positive | 0 | 2 | 4 | 36 | 100 % |
| Negative | 36 | 2 | 0 | 0 | 95 % |

Accuracy samples were categorized based upon the *d*-amphetamine GC/MS concentration only. The table below identifies those samples with a *d*-amphetamine concentration below the cutoff, in which the observed result on a COBAS INTEGRA 800 analyzer was positive. The expected results column identifies the result expected with the Amphetamines II assay based upon the *d*-amphetamine (AMP) value relative to the cutoff.

**GC/MS Summary of Discrepant Results (AMP)**

| Cutoff Value (ng/mL) | Roche ONLINE DAT AMPII OBSERVED Result | Roche ONLINE DAT AMPII EXPECTED Result | GC/MS (ng/mL) | Drug / Metabolite |
|---|---|---|---|---|
| 300 (SQ, Q) | Positive | Positive | 157<br>363 | AMP<br>MAMP |
| 300 (SQ, Q) | Positive | Positive | 181<br>173 | AMP<br>MAMP |
| 300 (SQ, Q) | Positive | Positive | 220<br>171 | AMP<br>MAMP |
| 500 (SQ, Q) | Positive | Positive | 438<br>121 | AMP<br>MAMP |

| Cutoff Value (ng/mL) | Roche ONLINE DAT AMPII OBSERVED Result | Roche ONLINE DAT AMPII EXPECTED Result | GC/MS (ng/mL) | Drug / Metabolite |
|---|---|---|---|---|
| 500 (SQ, Q) | Positive | Positive | 457<br>1152 | AMP<br>MAMP |
| 500 (SQ, Q) | Positive | Positive | 443<br>706 | AMP<br>MAMP |
| 1000 (SQ,) | Positive | Negative | 920 | AMP |
| 1000 (SQ, Q) | Positive | Positive | 837<br>1163 | AMP<br>MAMP |

**Analytical specificity**

The specificity of Amphetamines II, on the COBAS INTEGRA 800 analyzer, for various phenethylamines and structurally similar compounds was determined by generating inhibition curves for each of the compounds listed for both semiquantitative and qualitative modes and determining the approximate quantity of each compound that is equivalent in assay reactivity to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL *d*-methamphetamine assay cutoff. The tables below show the semiquantitative results of the study for each assay cutoff. The same samples were run in the qualitative mode and all recovered appropriately negative or positive, based on the calculated cross-reactivity.

| Compound | ng/mL Equivalent to 300 ng/mL MAMP | Approximate percent cross-reactivity |
|---|---|---|
| ± MDMA[c)] | 114 | 264 |
| PMA[d)] | 143 | 210 |
| PMMA[e)] | 181 | 166 |
| ± MDA[f)] | 249 | 121 |
| ± MDEA[i)] | 285 | 105 |
| *d* -Amphetamine | 311 | 96[g)] |
| *d* -Methamphetamine | 327 | 92 |
| ± MBDB HCl[h)] | 339 | 88 |
| ± BDB HCl[j)] | 648 | 46 |
| meta-Chlorphenylpiperazine[k)] | 1322 | 23 |
| 1-Methyl-3-phenylpropylamine[l)] | 1792 | 17 |
| *l*-Methamphetamine | 2754 | 11 |
| *l*-Amphetamine | 6443 | 5 |
| Dimethylamylamine[m)] | 21111 | 1.4 |
| Phendimetrazine | 47473 | 0.63 |
| Phentermine | 66385 | 0.45 |
| Tyramine | 86271 | 0.35 |
| *d*-Pseudoephedrine | 87578 | 0.34 |
| *l*-Ephedrine | 94792 | 0.32 |
| *d,l*-Phenylpropanolamine HCl | 280374 | 0.11 |
| *d*-Ephedrine | 329670 | 0.09 |

| Compound | ng/mL Equivalent to 500 ng/mL MAMP | Approximate percent cross-reactivity |
|---|---|---|
| ± MDMA[c)] | 173 | 289 |
| PMA[d)] | 231 | 216 |
| PMMA[e)] | 299 | 167 |
| ± MDA[f)] | 433 | 115 |
| *d* -Methamphetamine | 444 | 113 |
| *d* -Amphetamine | 460 | 109[g)] |
| ± MDEA[i)] | 494 | 101 |
| ± MBDB HCl[h)] | 713 | 70 |
| ± BDB HCl[j)] | 1209 | 41 |
| meta-Chlorphenylpiperazine[k)] | 2414 | 21 |
| 1-Methyl-3-phenylpropylamine[l)] | 2925 | 17 |
| *l*-Methamphetamine | 4098 | 12 |
| *l*-Amphetamine | 11174 | 4 |
| Phendimetrazine | 72500 | 0.69 |
| Phentermine | 118483 | 0.42 |
| Dimethylamylamine[m)] | 37701 | 1.3 |
| *d*-Pseudoephedrine | 132275 | 0.38 |
| Tyramine | 141243 | 0.35 |
| *l*-Ephedrine | 154321 | 0.32 |
| *d,l*-Phenylpropanolamine HCl | 390625 | 0.13 |
| *d*-Ephedrine | 413223 | 0.12 |

| Compound | ng/mL Equivalent to 1000 ng/mL MAMP | Approximate percent cross-reactivity |
|---|---|---|
| ± MDMA[c)] | 446 | 224 |
| PMA[d)] | 599 | 167 |
| PMMA[e)] | 695 | 144 |
| ± MDA[f)] | 785 | 127 |
| *d*-Methamphetamine | 970 | 103 |
| *d*-Amphetamine | 1024 | 98[g)] |
| ± MBDB HCl[h)] | 1194 | 84 |
| ± MDEA[i)] | 1203 | 83 |
| ± BDB HCl[j)] | 2262 | 44 |
| meta-Chlorphenylpiperazine[k)] | 4946 | 20 |
| 1-Methyl-3-phenylpropylamine[l)] | 5479 | 18 |
| *l*-Methamphetamine | 9008 | 11 |
| *l*-Amphetamine | 23445 | 4 |
| Dimethylamylamine[m)] | 79780 | 1.3 |
| Phendimetrazine | 156740 | 0.64 |
| *d*-Pseudoephedrine | 269542 | 0.37 |
| Phentermine | 294118 | 0.34 |
| *l*-Ephedrine | 317460 | 0.32 |
| Tyramine | 323625 | 0.31 |
| *d*-Ephedrine | 793651 | 0.13 |
| *d,l*-Phenylpropanolamine HCl | 1111111 | 0.09 |

c) *d,l*-3,4-Methylenedioxymethamphetamine

d) para-Methoxyamphetamine

e) para-Methoxymethamphetamine

f) *d,l*-3,4-Methylenedioxyamphetamine

g) Representative data from multiple lots demonstrate cross-reactivity in the range from approximately 75-125 %

h) *d,l*-N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine hydrochloride

i) *d,l*-3,4-Methylenedioxyethylamphetamine

j) *d,l*-3,4-Methylenedioxyphenyl-2-butanamine hydrochloride
k) 1-(3-Chlorphenyl)piperazine (mCPP), major metabolite of Trazodone
l) APB, metabolite of Labetalol
m) 4-Methylhexan-2-amine, DMAA

**Interference and cross-reactivity with unrelated drugs**

The following compounds were added at the listed concentrations to a human urine pool spiked with either *d*-amphetamine or *d*-methamphetamine at approximately the negative and positive control concentrations for each cutoff (± 25 % of assay cutoff). For each compound, the control level samples recovered properly for the 300 ng/mL, 500 ng/mL, and 1000 ng/mL cutoff in both semiquantitative and qualitative modes.

| | | Semiquantitative All Cutoffs | | Qualitative All Cutoffs | |
|---|---|---|---|---|---|
| Compound | Conc. (ng/mL) | Low Ctrl | High Ctrl | Low Ctrl | High Ctrl |
| Acetaminophen | 100000 | NEG | POS | NEG | POS |
| Acetylsalicylic acid | 100000 | NEG | POS | NEG | POS |
| Amitriptyline | 100000 | NEG | POS | NEG | POS |
| Aspartame | 40000 | NEG | POS | NEG | POS |
| Benzocaine | 100000 | NEG | POS | NEG | POS |
| Benzoylecgonine | 100000 | NEG | POS | NEG | POS |
| Caffeine | 100000 | NEG | POS | NEG | POS |
| Cannabidiol | 100000 | NEG | POS | NEG | POS |
| Cocaine | 100000 | NEG | POS | NEG | POS |
| Codeine | 100000 | NEG | POS | NEG | POS |
| Desipramine HCl | 100000 | NEG | POS | NEG | POS |
| Dextromethorphan | 100000 | NEG | POS | NEG | POS |
| Dextropropoxyphene | 100000 | NEG | POS | NEG | POS |
| Diazepam | 100000 | NEG | POS | NEG | POS |
| Digoxin | 100000 | NEG | POS | NEG | POS |
| Diphenhydramine | 100000 | NEG | POS | NEG | POS |
| Diphenylhydantoin | 100000 | NEG | POS | NEG | POS |
| Doxepin | 100000 | NEG | POS | NEG | POS |
| Ecgonine | 100000 | NEG | POS | NEG | POS |
| Ecgonine methyl ester | 100000 | NEG | POS | NEG | POS |
| Erythromycin | 100000 | NEG | POS | NEG | POS |
| Furosemide | 100000 | NEG | POS | NEG | POS |
| Guaiacol glycerol ether | 100000 | NEG | POS | NEG | POS |
| Hydrochlorothiazide | 100000 | NEG | POS | NEG | POS |
| Ibuprofen | 100000 | NEG | POS | NEG | POS |
| Ketamine | 100000 | NEG | POS | NEG | POS |
| Levothyroxine | 100000 | NEG | POS | NEG | POS |
| LSD | 2500 | NEG | POS | NEG | POS |
| Meperidine | 100000 | NEG | POS | NEG | POS |
| Methadone | 100000 | NEG | POS | NEG | POS |
| Methaqualone | 75000 | NEG | POS | NEG | POS |
| Morphine | 100000 | NEG | POS | NEG | POS |
| Naloxone | 100000 | NEG | POS | NEG | POS |
| Naltrexone | 100000 | NEG | POS | NEG | POS |
| Naproxen | 100000 | NEG | POS | NEG | POS |
| Niacinamide | 100000 | NEG | POS | NEG | POS |
| Nicotine | 100000 | NEG | POS | NEG | POS |
| Nifedipine | 100000 | NEG | POS | NEG | POS |
| Nordiazepam | 100000 | NEG | POS | NEG | POS |
| Omeprazole | 100000 | NEG | POS | NEG | POS |
| Oxazepam | 100000 | NEG | POS | NEG | POS |
| Penicillin G | 100000 | NEG | POS | NEG | POS |
| Phencyclidine | 40000 | NEG | POS | NEG | POS |
| Phenobarbital | 100000 | NEG | POS | NEG | POS |
| Quinine | 100000 | NEG | POS | NEG | POS |
| Secobarbital | 100000 | NEG | POS | NEG | POS |
| Tetracycline | 100000 | NEG | POS | NEG | POS |
| $\Delta^9$-THC | 10000 | NEG | POS | NEG | POS |

The compounds, including methylphenidate (Ritalin), were additionally added to aliquots of pooled drug-free 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 0.19 % cross-reactivity and no results were greater than the assay cutoffs (300 ng/mL, 500 ng/mL, and 1000 ng/mL), with the following exception.

The compounds Labetalol HCl and Trazodone were additionally added to aliquots of pooled drug-free human urine at a concentration of 100000 ng/mL. Ranitidine was added to aliquots of pooled drug-free human urine at a concentration of 50000 ng/mL. The results obtained were between 0.21 % and 0.36 % for the 300 ng/mL, 500 ng/mL and the 1000 ng/mL assay cutoffs respectively.

The cross-reactivity for LSD was tested at a concentration of 2500 ng/mL. The results obtained were 0.32 % and 0.71 %, for the 300 ng/mL and 1000 ng/mL assay cutoffs respectively.

The cross-reactivity for procaine HCl was tested in drug-free urine at a concentration of 100000 ng/mL. The results obtained were 1.00 %, 0.99 %, and 0.90 %, for the 300 ng/mL, 500 ng/mL, and 1000 ng/mL assay cutoffs respectively.

**Interference with other substances**

Interfering substances were added to urine containing *d*-methamphetamine (MAMP) at –25 % and +25 % of the cutoff level at the concentration listed below. The same substances were additionally added to urine containing *d*-amphetamine (AMP) at –25 % and +25 % of the cutoff level at the concentration listed below. All samples were tested and the following results were obtained on a COBAS INTEGRA 800 analyzer. The value in the table indicates the level at which no interference was found for samples containing either *d*-methamphetamine or *d*-amphetamine.

| Qualitative | | 300 ng/mL Cutoff | | 500 ng/mL Cutoff | | 1000 ng/mL Cutoff | |
|---|---|---|---|---|---|---|---|
| Compound | Cmpd. Conc. | Neg Level | Pos Level | Neg Level | Pos Level | Neg Level | Pos Level |
| Acetone | 7.9 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Ascorbic Acid | 10 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Conjugated Bilirubin | 0.1 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Creatinine | 5 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Ethanol | 7.9 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Glucose | 12 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Hemoglobin | 1 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Human serum albumin | 3 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Oxalic Acid | 2 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Sodium Chloride | 23 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |
| Urea | 60 mg/mL | Neg | Pos | Neg | Pos | Neg | Pos |

The same experiment was performed in the semiquantitative mode for each cutoff. All negative and positive controls recovered properly in the presence of the interfering substance.

A protocol was executed in which samples containing either AMP or MAMP at control levels (± 25 % of cutoff) with specific gravities ranging from 1.001 to 1.034 were tested. As with the other interferences, there were no control cross-overs on any of the 3 assay cutoffs at either extreme specific gravity level. Samples having a pH ranging from 4.5 to 8.0 and containing either AMP or MAMP at control levels (± 25 % of cutoff) were also tested. There were ≤ 5 % cross-overs on any of the 3 assay cutoffs.

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

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**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 |

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.

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