**DRI® Methadone Metabolite Assay**

**For In Vitro Diagnostic Use** (100 mL Kit)

This procedure is valid for the following chemistry analyzer: Beckman AU 5800

**PRINCIPLE**

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

Once methadone is administered, it is quickly metabolized by the liver to normethadone by N-demethylation. Normethadone is rarely detected because it readily dehydrates to form EDDP (2-ethylidene-1, 5- dimethyl-3, 3-diphenylpyrrilodine), the primary metabolite of methadone. EDDP undergoes further demethylation to form EMDP ( 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline), the secondary metabolite of methadone which is present in lower concentrations.

False positive results can be obtained in urine with various methadone immunochemical techniques when an addict adds a portion of their methadone directly into their urine sample. The determination of EDDP in urine with an immunoassay allows for more accurate assessment of patient’s methadone compliance.

The methadone assay utilizes liquid ready to use reagents and calibrators, the assay uses specific antibodies that can detect EDDP in human urine without cross reactivity to the parent drug, methadone. The assay is based on competition between a drug labeled with glucose-6-phospate dehydrogenase(G6PDH), and free drug from the urine sample for a fixed amount of specific antibody binding sites, In the absence of free drug from the sample, the specific antibodies binds.

**INTENDED USE**

The DRI® Methadone Metabolite Assay is an in vitro diagnostic medical device intended for the qualitative and semi quantitative determination of Methadone Metabolite, (2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrilodine or EDDP) in human urine at a cutoff level of 1000 ng/mL. This assay is intended as a preliminary analytical test result to aid in the detection of methadone metabolites, A confirmed analytical result on Gas chromatography/mass spectrometry is the preferred confirmatory method. Analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reaction accurately can be used to perform this assay. Refer to the specific application instructions of each analyzer for chemistry parameters before performing the assay. This assay has been validated for use on the Beckman 5800 analyzer.

**METHODOLOGY**

The DRI Methadone Metabolite Assay is a liquid, ready-to-use homogeneous enzyme immunoassay. The assay is based on competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme and free drug from the urine sample for a fixed amount of specific antibody binding sites. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

**SPECIMEN**

## Patient / Sample Preparation:

None required.

## Type:

Urine samples are the recommended specimen type.

Sample volume: 13.9 uL

## Handling Conditions:

Urine specimens may be collected in plastic (i.e., polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics, other than those listed, can adsorb certain drugs.

Testing of fresh urine specimens is suggested.

If not analyzed immediately, specimens may be stored at room temperature (15-25°C) for up to 7 days following collection. After 7 days, specimens should be refrigerated at 2-8°C

Foe two months and longer frozen (< -20°C). Frozen specimens must be completely thawed, mixed thoroughly, and centrifuged prior to analysis.

Samples with a pH range from 4 to 9 are suitable for testing with this assay.

Specimens with high turbidity should be centrifuged before analysis.

Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain another specimen.

Human urine specimens should be handled and treated as if they are potentially infectious.

## Criteria for Unacceptable Specimens

The sample must be properly labeled with the minimum of the patient’s name and date of birth.

For more information on the acceptability of samples, see Specimen Rejection Policy in the Administrative Manual.

**EQUIPMENT AND MATERIALS**

**Equipment:**

Beckman Coulter AU 5800

**Materials:**

Reagents, calibrators, and controls are stored in the Chemistry refrigerator. All assay components, when stored refrigerated, are stable until the expiration date indicated on the label.

**Preparation:**

The reagents are ready for use. No reagent preparation is required.

The calibrators are ready to use and are prepared from methadone metabolite spiked human urine.

The controls are ready to use and are prepared from EDDP- spiked human urine at levels of 750 ng/mL and 1250 ng/mL.

***Antibody/Substrate Reagent:*** Contains mouse monoclonal anti -EDDP antibody, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.

***Enzyme Conjugate Reagent:*** Contains EDDP labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as preservative.

 ***Additional Materials Required (sold separately):***

**Kit Description**  DRI Methadone Metabolite Calibrators : 150 ng/ml,300 ng/ml, 1000 ng/ml, 2000 ng/ml and negative urine Calibrator. MGC Primary DAU control set with an EDDP low range at 750 ng/ml and 1250 ng/ml.

Test tubes 12 -16 mm in diameter or sample cups (Cat No. AU1063).

Sample tubes and cups are stored in the Main Chemistry area.

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**Precautions and Warnings**

This test is for in vitro diagnostic use only.

The reagents, calibrators and controls are harmful if swallowed or inhaled.

Reagents used in the assay components contain < 0.09% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide. Do not use the reagents beyond their expiration dates.

**PERFORMANCE PARAMETERS**

Typical manufacturer performance results obtained on the Hitachi 717 analyzer are shown below.10 **The results obtained on the Beckman AU 5800 are available on site in the validation manual.**

 ***Precision***

 The cut off calibrator (1000 ng/ml) , the controls (750 ng/ml and 1250 ng/ml) were tested using a modified NCCLS protocol. The test was run in qualitative mode by testing all three levels in replicates of 6, twice per day for 10 days.

***Qualitative (mA/min)***

|  |  |  |
| --- | --- | --- |
| ***CAL/QC*** | ***WITHIN RUN PRECISION*** | ***TOTAL PRECISION*** |
| ***(n=20)*** | ***MEAN*** | ***SD*** | ***%CV*** | ***MEAN*** | ***SD*** | ***%CV*** |
| ***750 ng/ml*** | ***763*** | ***19.7*** | ***2.6*** |  ***763*** | ***22.1*** | ***2.9*** |
| ***1000*** ***ng/ml*** | ***1016*** | ***23.6*** | ***2.3*** | ***1016*** | ***25.7*** | ***2.5*** |
| ***1250 ng/ml*** | ***1270*** | ***34.7*** | ***2.7*** | ***1270*** | ***36.8*** | ***2.9*** |

***Accuracy***

One hundred and fifty clinical samples were analyzed by the DRI Methadone Metabolite Assay obtained from patients receiving methadone treatment . All the samples were confirmed by GC/MS. The results obtained by qualitative mode are summarized below:

***Qualitative***

Concordance agreement was greater than 95%.:

 DRI Metabolite Assay

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 GC/MS + 69 7

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**CALIBRATION**

***Qualitative analysis***

For qualitative analysis of samples, use the 1000 ng/mL calibrator as a cutoff level. The DRI® Methadone Metabolite Calibrator, which contains 1000 ng/mL cotinine, is used as a cutoff reference for distinguishing “positive” and “negative” samples. The calibration and blanking of the assay will be performed every 3 days.

**Results and Expected Values- *Qualitative results***

The 1000 ng/mL calibrator is used as a Cutoff reference for distinguishing “positive” from “negative” samples. A sample that exhibits a change in absorbance (ΔA) value equal to or greater than the value obtained with the cutoff calibrator is considered positive. A sample that exhibits a change in absorbance (ΔA) value lower than the value obtained with the cutoff calibrator is considered negative.

**QUALITY CONTROL**

During operation of the Beckman Coulter AU analyzer at least two levels of control material should be tested a minimum of once a day. Controls should be performed after calibration, with each new lot/bottle of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User’s Guide. Quality control testing should be performed in accordance with regulatory requirements and individual laboratory’s standard procedures. If more frequent verification of test results is required by the operating procedures within your laboratory, those requirements should be met.

## Qualitative Analysis

Validate the calibration by assaying controls. Ensure that the result from the negative control is negative (or lower) relative to the Calibrator/ Control set point. Ensure that the result from the positive is positive (or higher) relative to the Calibrator/Control set point. Once the calibration is validated, run urine specimens.

**PARAMETERS**

A complete list of test parameters and insert updates can be found at [www.thermoscientific.com](http://www.thermoscientific.com).

**CALCULATIONS**

None required.

**REPORTING RESULTS**

## Reference Ranges:

No reference ranges are defined for drugs of abuse testing.

## Procedures for Abnormal Results:

Abnormal results are flagged by the Beckman AU 5800 analyzer according to the normal values entered by the user into the instrument parameters.

## Reporting Format:

**Negative** values are resulted as None Detect.

**Positive** values will be resulted as POS SCREEN (positive not confirmed). Toxicology may change results to POSITIVE when confirmatory tests are performed.

Numerical value with flag from AU-report as **Inconclusive** with comment:

*Unable to result due to elevated absorbance readings. This may indicate an interfering substance. If confirmatory testing is desired, contact the Toxicology lab within 5 days of sample collection.*

***Interpretation of Results***

**Qualitative Analysis** -- When the DRI® Methadone Metabolite Assay is used as a qualitative assay, the amount of drug and metabolites detected by the assay in any given sample **cannot** be estimated. The assay results distinguish positive from negative samples only. The Calibrator/Control Cutoff as designated by the testing facility, which contains a concentration of 1000 ng/mL, is used as a reference for distinguishing “positive” from “negative” specimens.

**Positive Results:**A specimen that gives a result equal to or higher than the Calibrator/Control set point is interpreted as positive: The specimen contains methadone metabolite.

**Negative Results:**A specimen that gives a result lower than the Calibrator/Control set point is interpreted as negative. Either the specimen does not contain methadone metabolite or methadone metabolites are present in concentrations below the cutoff level for this assay.

**LIMITATIONS**

1. A positive result from this assay indicates only the presence of EDDP and does not necessarily correlate with the extent of physiological and psychological effects.

2. It is possible that other substances and/or factors, e.g., technical, procedural issues or other than those investigated in the specificity study, may interfere with the test and cause false results.

***Sensitivity***

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

***Specificity***

The specificity of the assay was evaluated by testing parent drug and its metabolites. Other compounds that are commonly encountered in urine samples were also tested.Methadone ans it’s metabolites produced a negative result at the concentrations below:

|  |  |  |
| --- | --- | --- |
| COMPOUND | CONCENTRATION ng/ml | RESULT |
| Methadone | 35,000,000 |  Negative  |
| EMDP |  200,000 | Negative |
| LAAM-HCL |  100,000 | Negative |
| Nor-LAAM-HCL |  100,000 | Negative |
|  |  |  |

***Specificity cont’d***

Compounds structurally **unrelated** to methadone metabolites produced negative results at the concentrations listed below:

|  |  |
| --- | --- |
| COMPOUND | CONCENTRATION ng/ml |
| Acetaminophen | 1,000,000 |
| Acetylsalicylic acid | 1,000,000 |
| Amphetamine |  1,000,000 |
| Benzoylecgonine |  1,000,000 |
| Caffeine | 100,000 |
| Captopril | 500,000 |
| Chlordiazepoxide | 100,000 |
| Cimetidine | 500.000 |
| Cocaine | 200,000 |
| Codine | 1,000,000 |
| Dextromethorphan | 300,000 |
| Diazepam | 100,000 |
| Diphenhydramine | 500,000 |
| Disopyramide | 1,000,000 |
| Doxylamine | 500.000 |
| Ephedrine | 1,000,000 |
| Fluoxetine | 500,000 |
| Ibuprofen | 500.000 |
| Ketamine |  1,000,000 |
| Levothyroxine | 500,000 |
| Meperidine | 1,000.000 |
| d-Methamphetamine | 100,000 |
| l-Methamphetamine | 100,000 |
| Morphine | 1,000,000 |
| Oxazepam | 500,000 |
| Phencyclidine | 500,000 |
| Phenobarbital | 1,000,000 |
| Phentermine | 1,000,000 |
| Promethazine | 100,000 |
| Propoxyphene | 1,000,000 |
| Ranitidine | 500,000 |
| Salicyluric Acid | 500,000 |
| Secobarbital | 1,000,000 |
| Nor-Δ9-THC-9-COOH | 1,000,000 |

***Interference***

Endogenous and exogenous substances were studied for potential interference with the Methadone Metabolite Assay. No interference was observed in urine samples containing compounds at the concentrations listed below. Urine pH was also studied for possible interference.

|  |  |
| --- | --- |
| COMPOUND | CONCENTRATION  |
| Acetaminophen | 100 μg/mL |
| Acetone | 1000 mg/dL |
| Ascorbic Acid | 1000 mg/dL |
| Aspirin | 100 μg/mL |
| Caffeine | 100 μg/mL |
| Creatinine | 500 mg/dL |
| Ethanol | 1 g/dL |
| Galactose | 10 mg/dL |
| Gamma Globulin | 500 mg/dL |
| Glucose | 3000 mg/dL |
| Hemoglobin | 150 mg/dL |
| Human serum Albumin | 500 mg/dL |
| Ibuprofen | 100 μg/mL |
| Oxalic Acid | 100 mg/dL |
| Riboflavin | 7.5 mg/dL |
| Sodium Chloride | 1 g/dL |
| Urea | 1.25 g/dL |
| pH range | 3-11 |

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14. Data on traceability are on file at Microgenics, a part of Thermo Fisher Scientific. 15. Data on file at Microgenics, a part of Thermo Fisher Scientific.