DRI® Methadone Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10016403 (3 x 18 mL Kit)
0596 (100 mL Kit)
0597 (500 mL Kit)

Intended Use
The DRI® Methadone Assay is intended for the qualitative and semiquantitative determination of methadone in human urine.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1,2] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary and Explanation of the Test
Methadone, a synthetic opioid, has been used in the treatment of heroin addiction. Methadone compliance is essential and can be effectively monitored by urine screening for methadone or its metabolite.

When methadone is ingested, it is rapidly metabolized in the liver. The primary methadone metabolite is formed by N-demethylation to normethadone. However, normethadone is rarely detected as it readily dehydrates to form 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine, commonly known as EDDP.[3,4] Further demethylation of EDDP forms 2-ethyl-5-methyl-3, 3-diphenyl-1-pyrroline (EMDP) which is the secondary metabolite of methadone.

Various techniques including TLC, GC and immunoassays are available for methadone compliance monitoring.[5] Both TLC and GC methods[6] are laborious and subject to interference. Immunoassays can be easily performed with an automated clinical chemistry analyzer. Determination of the presence of methadone in urine with an immunoassay will make widespread testing for compliance possible.

The DRI Methadone Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents.[7] The assay uses specific antibodies, which can detect methadone in urine. The assay is based on the competition of an enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled drug and the drug from the urine sample for a fixed amount of specific antibody binding sites. In the absence of drug from the sample, the specific antibody binds to the drug labeled with G6PDH and the enzyme activity is inhibited. This phenomenon creates a direct relationship between the drug concentration in the urine and the enzyme activity. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

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

Additional Materials Required (sold separately):

REF	Kit Description
1664	DRI Negative Calibrator, 10 mL
1388	DRI Negative Calibrator, 25 mL
1588	DRI Multi-Drug Calibrator 1, 10 mL
1589	DRI Multi-Drug Calibrator 1, 25 mL
1591	DRI Multi-Drug Calibrator 2, 10 mL
1592	DRI Multi-Drug Calibrator 2, 25 mL
1594	DRI Multi-Drug Calibrator 3, 10 mL
1595	DRI Multi-Drug Calibrator 3, 25 mL
1597	DRI Multi-Drug Calibrator 4, 10 mL
1598	DRI Multi-Drug Calibrator 4, 25 mL
100200	MGC Primary DAU Control Set, 3 x 5 mL each (high and low)

⚠ Precautions and Warning
This test is for in-vitro diagnostic use only. The components are harmful if swallowed.

DANGER: Reagents used in the assay components contain ≤0.09% sodium azide, ≤0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody. 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.
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Avoid breathing mist or vapor. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/ face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Do not use the reagents beyond their expiration dates.

Reagent Preparation and Storage
The reagents are ready for use. No reagent preparation is required. All assay components, when stored at 2-8°C, are stable until the expiration date indicated on the label.

Specimen Collection and Handling
Collect urine specimens in plastic or glass containers. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed.

Specimens kept at room temperature that do not receive initial test within 7 days[8] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for up to thirty days.[8] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[9, 10]

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

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[11]

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. An effort should be made to keep pipetted samples free of gross debris. It is recommended that grossly turbid specimens be centrifuged before analysis. Frozen samples should be thawed and mixed prior to analysis. Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample and forward both specimens to the laboratory for testing.

Handle all urine specimens as if they were potentially infectious.

Assay Procedure
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.

Quality Control and Calibration
Qualitative analysis
For qualitative analysis of samples, use the 300 ng/mL calibrator as a cutoff level. The DRI Calibrator 2, which contains 300 ng/mL methadone, is used as a cutoff reference for distinguishing "positive" and "negative" samples.

Semiquantitative analysis
For semiquantitative analysis, use all calibrators.

Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Use controls near the cutoff calibrator to validate the calibration. Control results must fall within the established range. If results fall outside of the established range, assay results are invalid. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values
Qualitative results
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.

Semiquantitative results
A rough estimate of drug concentration in the samples can be obtained by running a standard curve with all calibrators and quantitating samples off the standard curve.

Limitations
1. A positive result from this assay indicates only the presence of methadone and does not necessarily correlate with the extent of physiological and psychological effects.
2. A positive result by this assay should be confirmed by an other nonimmunological method such as GC, GC/MS or TLC.
3. The test is designed for use with human urine only.
4. It is possible that other substances and/or factors (eg, technical or procedural) not listed in the specificity table may interfere with the test and cause false results.

Typical Performance Characteristics

Performance data results obtained on the Hitachi 717 analyzer are shown below.[12] The results obtained in your laboratory may differ from these data.

Precision

The Negative, 300 ng/mL calibrator and 1000 ng/mL calibrator were assayed, and the following results were obtained:

Qualitative:

Calibrator	Within-run (n=20)		Run-to-run (n=12)	
	Mean ± SD (mA/min)	%CV	Mean ± SD (mA/min)	%CV
Negative	258 ± 1.3	0.5	258 ± 1.3	0.5
300 ng/mL	340 ± 2.4	0.7	340 ± 2.9	0.8
1000 ng/mL	472 ± 2.4	0.5	472 ± 4.6	0.9

Semiquantitative :

Control	Within-run (n=20)		Run-to-run (n=12)	
	Mean ± SD (ng/mL)	%CV	Mean ± SD (ng/mL)	%CV
Control 1	166 ± 4.5	2.7	166 ± 5.7	3.4
Control 2	403 ± 6.8	1.7	406 ± 7.1	1.7

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 10 ng/mL.

Accuracy

Ninety-six clinical urine specimens were tested with a commercially available methadone assay and DRI Methadone Assay. There was 100% agreement between the two methods. Forty-six samples were positive and fifty were negative by both assays. In addition, all forty-six positive samples were confirmed positive by the GC/MS method.

Specificity

Various potentially interfering substances were tested for cross-reactivity with the assay. Table 1 lists the compounds producing a positive result at the concentration listed. Table 2 lists the compounds producing a negative result at the concentration listed.

Table 1

Compound	Concentration Tested (ng/mL)
Methadone	300
Methadol	750

Table 2

Compound	Concentration Tested (ng/mL)
1-α-Acetylmethadol (LAAM)	5,000
Acetaminophen	1,000,000
Acetylsalicylic acid	1,000,000
Amitriptyline	50,000
Amphetamine	1,000,000
Benzoylecgonine	400,000
Caffeine	100,000
Carbamazepine	20,000
Cocaine	200,000
Codeine	500,000
Dextromethorphan	250,000
Diphenhydramine	1,000,000
Ephedrine	1,000,000
Imipramine	50,000
Meperidine	150,000
Methadone Metabolite (EDDP)	10,000
Methadone Metabolite (EMDP)	10,000
Morphine	200,000
Nortriptyline	50,000
Orphenadrine	1,000,000
Oxazepam	500,000
Phencyclidine	500,000
Phenobarbital	1,000,000
Phenytoin	40,000
Primidone	24,000
Promethazine	100,000
Propoxyphene	250,000
Secobarbital	1,000,000
Theophylline	50,000
Valproic Acid	150,000
Verapamil	1,000,000

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12. Data on file at Microgenics, a part of Thermo Fisher Scientific.

Glossary:

http://www.thermofisher.com/symbols-glossary

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