DXC (METD) METHADONE

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PURPOSE

To provide instructions for the qualitative determination of methadone on the DXC 600/800.

PRINCIPLE

METD reagent, in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Drugs of Abuse Testing (DAT) Urine Calibrators, is intended for the qualitative determination of Methadone in human urine at a cutoff value of 300 ng/mL.
The METD assay provides a rapid screening procedure for determining the presence of Methadone in urine. This test provides only a preliminary analytical result; a positive result by this assay should be confirmed by another generally accepted non-immunological method such as thin layer chromatography (TLC), gas chromatography (GC), or gas chromatography/mass spectrometry (GC/MS). GC/MS is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

BACKGROUND

Clinical Significance

Methadone is an addictive narcotic drug which is used for pain relief. Measurements of methadone are used in the diagnosis and treatment of methadone use and overdose, and to determine compliance with regulations in methadone maintenance treatment.

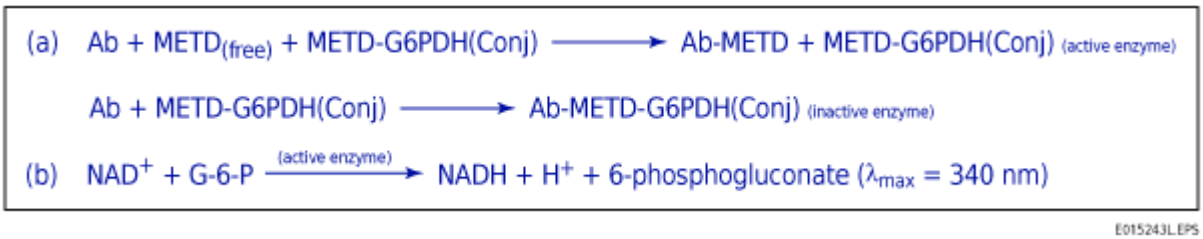
General Discussion

Methadone is a synthetic opioid used in the treatment of heroin addiction. Though intended for legitimate treatment of substance abuse, the potential for its illicit use exists. Methadone is rapidly metabolized after ingestion to its primary metabolite, normethadone. Normethadone, however, is rarely detected as it is readily dehydrated to form 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). EDDP is further demethylated to form 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP), the secondary metabolite of methadone. Methadone treatment compliance is essential and can be effectively monitored by urine screening of methadone or its metabolites. Confirmation of "false positives" from addicts who add a portion of their methadone directly into the urine sample is often required by detecting the presence of major metabolites using TLC or GC analysis.

Methodology

The Methadone assay utilizes a homogenous enzyme immunoassay method. The METD reagent is comprised of specific antibodies which can detect methadone in urine. A drug-labeled glucose-6-phosphate dehydrogenase (G6PDH) conjugate competes with any free drug from the urine sample for a fixed amount of antibody binding sites. In the absence of free drug from the sample, the drug-labeled G6PDH conjugate is bound by the specific antibody and enzyme activity is inhibited. This reaction creates a direct relationship between the presence of drug and enzyme activity. The G6PDH enzyme activity is determined spectrophotometrically by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH (reduced form).

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio for METD is one part sample to 25 parts reagent. The System monitors the change in absorbance at 340 nanometers to calculate and express a reaction rate. A qualitative result is reported based on a comparison of the sample rate to the calibrated cutoff rate.



RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
M-F-CH-0820	Chemistry Controls
J-F-CH-0826	DXC 800 Calibrators
M-F-CH-0826	Chemistry Calibrators

SPECIMEN

Type of Specimen

Freshly collected urine samples should be used for testing. Collect urine samples in glass or plastic (i.e., polypropylene, polycarbonate, polyethylene) containers. Urine samples should be collected in the manner routinely used for drug screening analysis. Samples should be at room temperature for testing.

Specimen Storage and Stability

If the sample cannot be analyzed immediately, it may be stored at +2°C to +8°C for up to 7 days. If longer storage is required or when a split sample collection method is used, samples should be stored frozen at -20°C or less.

Sample Type	Volume	Sample Stability
Urine	0.5mL	• Analyze at Room Temp • Refrigerated 7 days • Frozen (-20ºC) >7 days

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
One METD Reagent Cartridge (1 x 250 tests)

Volume per Test	
Sample Volume	10 uL
Total Reagent Volume	250 uL
Cartridge Volumes	A 200 uL B 50 uL C – –

Reactive Ingredients	
Antibody/Substrate Reagent: Monoclonal anti-methadone antibody (mouse) Glucose-6-phosphate (G6P) Nicotinamide adenine dinucleotide (NAD) Tris buffer	69mL
Enzyme Conjugate Reagent: Glucose-6-phosphate dehydrogenase (G6PDH) labeled with methadone derivative Tris buffer	18mL

Also non-reactive chemicals necessary for optimal system performance.

Reagent Preparation

No preparation is required.

Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria. Refer to the Quality Control section of this chemistry information sheet for Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines.

Reagent Storage and Stability

METD Reagent when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the cartridge label. Once opened, the reagent is stable for 90 days at +2°C to +8°C. Do not use beyond the manufacturer's expiration date. DO NOT FREEZE.

CALIBRATION

Calibrator Required

SYNCHRON Systems DAT Negative Urine Calibrator (0 ng/mL methadone)
SYNCHRON Systems DAT Multi-Drug Low (cutoff) Urine Calibrator (300 ng/mL methadone)
SYNCHRON Systems DAT Multi-Drug High Urine Calibrator (1000 ng/mL methadone)

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

SYNCHRON® Systems Drugs of Abuse Testing (DAT) Urine Calibrators are stable until the expiration date printed on the calibrator bottles if stored capped in the original containers at +2°C to +8°C.

Calibration Information

1. The DAT assays require three levels of calibrators. The calibration measures the separation between calibrators to ensure reagent integrity. The calibration factor generated is non-functional for sample result calculation.

2. The system must have a valid calibrator cutoff value in memory before controls or patient samples can be run. The cutoff value for each DAT chemistry represents the mean reaction rate of the Low Calibrator, and is reported in mA/min units on patient and control reports. Cutoff values are stored in memory until the next successful calibration.

3. Under typical operating conditions the METD reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

4. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

5. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls & M-F-CH0820 Chemistry Controls

STEPS

1. If necessary, load the reagent onto the system.

2. After reagent load is completed, calibration may be required.

3. Program controls for analysis.

4. After loading controls onto the system, follow the protocols for system operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

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CALCULATIONS

The system performs all calculations internally to produce the final qualitative result, reported as POSITIVE or NEGATIVE. The qualitative result is based on a comparison of the sample rate to the calibrated cutoff rate; a sample rate greater than or equal to the cutoff rate is reported as POSITIVE. A POSITIVE result (≥300 ng/mL) from this assay indicates only the presence of METD and does not necessarily correlate with the extent of physiological and psychological effects. A NEGATIVE test result indicates that METD is either not present, or is present at levels below the cutoff threshold of the test.

PERFORMANCE CHARACTERISTICS

Reference Range

Sample Type	Normal
Urine	"Not Detected"

Analyte	Normal Results	Suspect Results (Adulterated)
Creatinine	>20 mg/mL	<20 mg/mL

If results for Creatinine are outside normal parameters for urine drug testing, **DIL1** comment will be appended to screen results.

Reporting results

Result	Reported As
Negative	"Not Detected"
Positive	"Detected"

Relative Sensitivity and Specificity

Eighty clinical urine specimens were collected and tested. One hundred percent agreement was obtained between the SYNCHRON LX System and the SYNCHRON CX7 DELTA. The cutoff value of the SYNCHRON Systems Methadone assay is 300 ng/mL.

Relative Sensitivity: 100%
Relative Specificity: 100%
Overall Agreement: 100%

LIMITATIONS

1. The test is designed for use with human urine only.

2. Do not dilute the urine samples since this is a qualitative assay. Dilution of samples may produce erroneous results.

3. Interference has been demonstrated from mefenamic acid, a nonopioid analgesic.

4. Adulteration of the urine sample may cause erroneous results. Alteration of a urine specimen may be detected by checking the appearance, temperature, pH, specific gravity, and creatinine levels of a sample. If adulteration is suspected, obtain another sample and forward both specimens to the laboratory for testing.

5. An effort should be made to keep pipetted samples free from gross debris. It is recommended that highly turbid specimens be centrifuged before analysis.

6. To ensure that carryover is not a factor when interpreting results of the METD assay, rerun any positive sample if the preceding sample is positive.

Cross Reactivity

Methadone metabolites and various potential interfering substances in a human urine matrix were tested for cross-reactivity with the SYNCHRON Systems METD assay. The following table summarizes the results obtained at the concentrations tested for each potential cross-reactant.[a]

Compound	Concentration (µg/mL)	Effect
Methadone (cutoff)	0.3	Positive
Methadol	0.75	Positive
Acetaminophen	1000	Negative
Acetylsalicylic Acid	1000	Negative
Albuterol	1000	Negative
Amitriptyline	50	Negative
d-Amphetamine	1000	Negative
Benzoylecgonine	400	Negative
Caffeine	100	Negative
Carbamazepine	20	Negative
Cocaine	200	Negative
Codeine	500	Negative
Dextromethorphan	250	Negative
Diphenhydramine	1000	Negative
Doxylamine	100	Negative
EDDP (Methadone Metabolite)[c]	10	Negative
EMDP (Methadone Metabolite)[d]	10	Negative
Ephedrine	1000	Negative
Imipramine	50	Negative
l-α-Acetylmethadol (LAAM)	5	Negative
Labetalol	1000	Negative
Meperidine	150	Negative
Methaqualone	100	Negative
Metronidazole	1000	Negative
Morphine	200	Negative
Naloxone	1000	Negative
Naltrexone	1000	Negative
Nortriptyline	50	Negative
Orphenadrine	1000	Negative
Oxazepam	500	Negative
Phencyclidine	500	Negative
Phenobarbital	1000	Negative
Phenothiazine	1000	Negative
Phenytoin	40	Negative
Primidone	24	Negative
Promethazine	100	Negative
Propoxyphene	250	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative

Compound	Concentration (µg/mL)	Effect
Sertraline	1000	Negative
Theophylline	50	Negative
Valproic Acid	150	Negative
Verapamil	1000	Negative

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

REFERENCES

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