DXC (AMPH) AMPHETAMINES

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PURPOSE

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

PRINCIPLE

AMPH reagent, when used 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 Amphetamines in human urine, at a cutoff value of 1000 ng/mL.
The AMPH assay provides a rapid screening procedure for determining the presence of Amphetamines (AMPH) and its metabolites 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

Amphetamines are a class of central nervous system stimulants. The most common amphetamines include d-amphetamine, d,l-amphetamine, and d-methamphetamine. Measurements of amphetamines on the SYNCHRON® System(s) are used in the diagnosis and treatment of amphetamine use and overdose, and in monitoring the presence of amphetamine to ensure appropriate therapy.

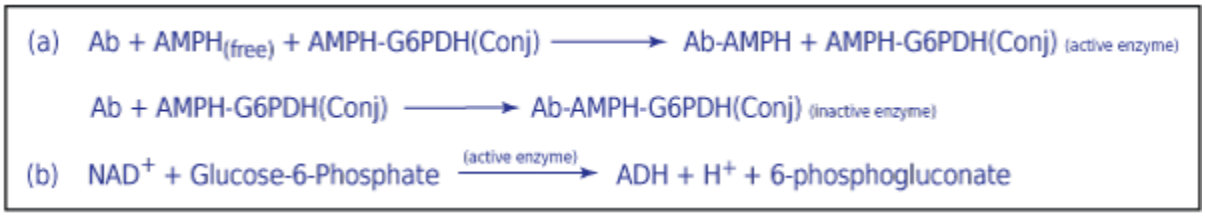
Amphetamines are synthetic derivatives of ephedrine. Due to their mood elevating properties, amphetamines are subject to widespread abuse, particularly in industrial societies. When amphetamine is ingested, it is readily absorbed in the GI tract and effects persist for 4 - 24 hours. Amphetamines appear in urine within about 3 hours following oral administration. Urinary excretion is pH-dependent and is enhanced in acidic pH.

Methodology

This assay utilizes a homogenous enzyme immunoassay method. The AMPH reagent is comprised of specific antibodies which can detect amphetamine and/or methamphetamine 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 AMPH is one part sample to 12.5 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.

Chemical Reaction



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 AMPH Reagent Cartridge (1 x 250 tests) Kit # 475000

Volume per Test		
Sample Volume	20 uL	
Total Reagent Volume	250 uL	
Cartridge Volumes	A 200 uL B 50 uL	Antibody/Substrate Reagent Enzyme conjugate Reagent

Reactive Ingredients	
Antibody/Substrate Reagent: Monoclonal anti-amphetamines antibodies (mouse) Glucose-6-phosphate (G6P) Nicotinamide adenine dinucleotide (NAD) Tris buffer	69mL
Enzyme Conjugate Reagent: Glucose-6-phosphate dehydrogenase (G6PDH) labeled with amphetamines Tris buffer Non reactive chemicals necessary for optimal system performance	18mL
β-glucuronidase (E. coli)	4mL

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

AMPH 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 manufacturers expiration date. DO NOT FREEZE.

CALIBRATION

Calibrator Required

SYNCHRON Systems DAT Negative Urine Calibrator (0 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug Low (cutoff) Urine Calibrator (1000 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug High Urine Calibrator (2000 ng/mL d-methamphetamine)

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.

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.
   NOTICE: the calibration factor generated is non-functional for sample result calculation.

3. Under typical operating conditions the AMPH 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. This assay has within-lot calibration available. Refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for information on this feature.

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 quality control 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.

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 (≥1000 ng/mL) from this assay indicates only the presence of this analyte and does not necessarily correlate with the extent of physiological and psychological effects. A NEGATIVE test result indicates that this analyte 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

One hundred twelve clinical urine specimens were collected and tested. 91% agreement was obtained between AMPH Reagent P/N 445965 (current) and improved AMPH Reagent P/N 475000 (new).
Six samples tested negative with the new reagent. Four samples, shown by GC/MS to contain ranitidine but no amphetamines, correctly tested negative with the new reagent, whereas they previously tested false positive.
Two samples testing negative with the new reagent, and positive with the current reagent, had rates within 0.8% and 1.3% of the cutoff rate (284 mA/min).
Four samples tested negative with the current reagent and positive with the new reagent. Two of the four had borderline rates (within 3.5% of the cutoff rate), and two contained phenylpropanolamine, pseudoephedrine and ephedrine.

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 nonopiod analgesic (which absorbs at 340 nm).

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. Interference may be caused by other substances and/or factors (e.g., technical or procedural errors) not listed above, producing false results.

CROSS REACTIVITY

Amphetamines, methamphetamines, amphetamine-like compounds, and various potential interfering substances in a human urine matrix were tested for cross-reactivity with the SYNCHRON Systems AMPH assay. The following table summarizes the results obtained at the concentrations listed for each potential cross-reactant.

Compound	Concentration (µg/mL)	Effect
d-Methamphetamine (cutoff)	1	Positive
d-Amphetamine	1	Positive
Methylenedioxyamphetamine (MDA)	2.5	Positive
Methylenedioxymethamphetamine (MDMA)	2.5	Positive
Acetaminophen	1000	Negative
Acetylsalicylic Acid	1000	Negative
l-Amphetamine	12.5	Negative
Benzoylecgonine	1000	Negative
Benzphetamine	20	Negative
Bupropion	50	Negative
Buspirone	1000	Negative
Caffeine	1000	Negative
Chlorpromazine	500	Negative
Codeine	1000	Negative
Dextromethorphan	1000	Negative
d-Ephedrine	400	Negative
d,l-Ephedrine	500	Negative
l-Ephedrine	350	Negative
Fenfluramine	4	Negative
3-Hydroxy-Tyramine	500	Negative
Isoxsuprine	100	Negative
Meperidine	1000	Negative
Mephentermine	25	Negative
Methadone	1000	Negative
l-Methamphetamine	10	Negative
Methapyrilene	500	Negative
Morphine	1000	Negative
Nor-pseudoephedrine	1000	Negative
Oxazepam	500	Negative

Compound	Concentration (µg/mL)	Effect
Phencyclidine	1000	Negative
Phendimetrazine	200	Negative
Phenethylamine	10	Negative
Phenmetrazine	50	Negative
Phenobarbital	1000	Negative
Phenothiazine	10	Negative
Phentermine	25	Negative
Phenylephrine	500	Negative
Phenylpropanolamine (PPA)	250	Negative
Procainamide	20	Negative
Promethazine	500	Negative
Propranolol	200	Negative
d-Pseudoephedrine	250	Negative
l-Pseudoephedrine	3000	Negative
Ranitidine	1000	Negative
Scopolamine	100	Negative
Secobarbital	1000	Negative
Setraline	1000	Negative
Thioridazine	1000	Negative
Trifluoperazine	1000	Negative
Triflupromazine	1000	Negative
Trazodone	1000	Negative
Tyramine	500	Negative

ADDITIONAL INFORMATION

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

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Updated for current process			
Committee Approval Date	☒ Date: 8/26/2014 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 8/26/14