BECKMAN COULTER CE

SYNCHRON System(s)

Chemistry Information Sheet

AMPH
Amphetamines

REF 475000

For *In Vitro* Diagnostic Use

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

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.[1,2]

Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

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.

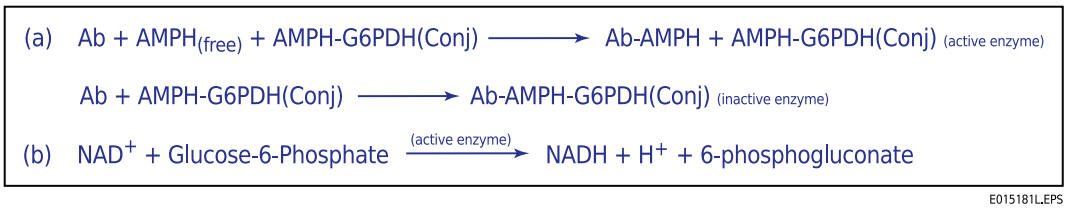
METHODOLOGY

This assay utilizes a homogenous enzyme immunoassay method.[3] 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 SCHEME



GENERAL DISCUSSION

Amphetamines are synthetic derivatives of ephedrine. Due to their mood elevating properties, amphetamines are subject to widespread abuse, particularly in industrial societies.[4] 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.[5]

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.[6] Samples should be at room temperature for testing.[7]

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.[2,6] If longer storage is required or when a split sample collection method is used, samples should be stored frozen at -20°C or less.[6]

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

One AMPH Reagent Cartridge (1 x 250 tests)

VOLUMES PER TEST

Sample Volume	20 µL	
Total Reagent Volume	250 µL	
Cartridge Volumes		
A	200 µL	Antibody/Substrate Reagent
B	50 µL	Enzyme Conjugate Reagent
C	– –	

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Antibody/Substrate Reagent	69 mL
Monoclonal anti-amphetamines antibodies (mouse)	
Glucose-6-phosphate (G6P)	
Nicotinamide adenine dinucleotide (NAD)	
Tris buffer	
Enzyme Conjugate Reagent	18 mL
Glucose-6-phosphate dehydrogenase (G6PDH) labeled with amphetamines	
Tris buffer	

Also non-reactive chemicals necessary for optimal system performance.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON Systems DAT Negative Urine Calibrator (0 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug Low Urine Calibrator (1,000 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug High Urine Calibrator (2,000 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug Low Urine Control (750 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug High Urine Control (1,250 ng/mL d-methamphetamine)

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 unless the expiration date is exceeded. DO NOT FREEZE.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON Systems DAT Negative Urine Calibrator (0 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug Low (cutoff) Urine Calibrator (1,000 ng/mL d-methamphetamine)
SYNCHRON Systems DAT Multi-Drug High Urine Calibrator (2,000 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.

⚠ CAUTION

Urine is not known to transmit infectious disease such as Hepatitis or HIV. However, because this product contains material of human origin, it should be handled as though capable of transmitting infectious diseases. The United States Food and Drug Administration recommends such samples be handled as specified in the Centers for Disease Control`s Biosafety Level 2 guidelines.[8]

Calibrator storage location:

CALIBRATION INFORMATION

1. The DAT assays require three levels of calibrators. The calibration measures the separation between calibrators to ensure reagent integrity.

NOTICE

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 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 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

Good laboratory practices suggest the use of control specimens to ensure proper assay performance. Each analytical run should include controls with levels 25% above and 25% below the cutoff threshold of each drug, as well as negative specimens certified to contain no drug.[9] In addition, these controls should be run with each new calibration, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

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

RESULTS INTERPRETATION

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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

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).[10]
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.[6] 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.

PERFORMANCE CHARACTERISTICS

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.

Table 2.0 Current Reagent vs. New Reagent[11]

AMPH		REAGENT P/N 475000		
		Positive	Negative	Total
REAGENT P/N 445965	Positive	44	6	50
	Negative	4	58	62
Total		48	64	112

Relative Sensitivity (% agreement among positives): 88%

Relative Specificity (% agreement among negatives): 94%

Overall Agreement: 91%

Forty-one positive samples were analyzed by GC/MS. Twenty-three samples were confirmed positive by GC/MS. Sixteen samples gave negative GC/MS results. Analysis of these samples showed the presence of phenylpropanolamine, ephedrine, and pseudoephedrine. These compounds are known potential cross reactants and are listed in Table 3.0 Cross Reactivity. One sample contained methadone and ranitidine and one sample contained amphetamine and methamphetamine at levels slightly below the GC/MS cutoff of 1,000 ng/mL.

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.

Table 3.0 Cross Reactivity[a]

COMPOUND	CONCENTRATION (µg/mL)	EFFECT
d-Methamphetamine (cutoff)	1	Positive
d-amphetamine	1	Positive
Methylenedioxyamphetamine (MDA)	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	200	Negative
L-Ephedrine	150	Negative

Table 3.0 Cross Reactivity, Continued

COMPOUND	CONCENTRATION (µg/mL)	EFFECT
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
Phencyclidine	1000	Negative
Phendimetrazine	200	Negative
Phenethylamine	10	Negative
Phenmetrazine	50	Negative
Phenobarbital	1000	Negative
Phenothiazine	10	Negative
Phentermine	25	Negative
Phenylephrine	300	Negative
Phenylpropanolamine (PPA)	250	Negative
Procainamide	20	Negative
Promethazine	500	Negative
Propranolol	200	Negative
d-Pseudoephedrine	250	Negative
l-Pseudoephedrine	2000	Negative
Ranitidine	1000	Negative
Scopolamine	100	Negative
Secobarbital	1000	Negative
Sertraline	1000	Negative
Thioridazine	1000	Negative
Trifluoperazine	1000	Negative
Trifluprомazine	1000	Negative
Trazodone	1000	Negative
Tyramine	500	Negative

a It is possible that other substances and/or factors (e.g. technical or procedural) not listed above may interfere with the test and cause false results. Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

PRECISION

The following estimates of within-run imprecision were obtained when 20 replicates of the Negative Calibrator, Control 1 (750 ng/mL), Calibrator 1 (1,000 ng/mL), Control 2 (1,250 ng/mL), and Calibrator 2 (2,000 ng/mL) were assayed on a properly operated and maintained SYNCHRON LX System.

Table 4.0 Typical Within-Run Imprecision

SAMPLE	MEAN RATE (mA/min)	1 SD (mA/min)	% CV
Negative Cal	185	0.9	0.5
Control 1	271	1.7	0.6
Cal 1	291	1.5	0.5
Control 2	308	1.7	0.5
Cal 2	330	2.0	0.6

All of the negative calibrator and low control rates were negative. All of the high calibrator and high control rates were positive. The low (cutoff) calibrator rates were uniformly distributed around the cutoff rate. Each laboratory should characterize their own instrument performance for comparison purposes. Instruments operated and maintained according to the manufacturer`s instructions should exhibit a within-run coefficient of variation of ≤ 2.0% for all sample levels.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

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

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REVISION HISTORY

Revision AE

Revised Quality Control section, and removed the sodium azide warning.

Revision AF

Updated corporate address.

Revision AG

Added Revision History

Revision AH

Added new language requirement: Czech, and Korean.

Revision AJ

Removed references to CX and LX systems as they are discontinued effective 12/2013.

Added Beckman Coulter trademark statement and disclaimer.

Revision AK

Added GHS Classification information

Revision AL

Updates to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AM

Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AN

Revised Cross Reactivity Section. Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AP

Revised Cross Reactivity Section.

Revision AR

Added new language requirement: Bulgarian, Serbian, and Vietnamese.

SYMBOLS KEY

Table 5.0

REF	Catalogue Number	IVD	In Vitro Diagnostic
CONTENTS	Contents		Temperature limit
	Manufacturer		Expiration Date
LOT	Batch code	SDS	Safety Data Sheet
CE	CE Mark		Consult Instructions for Use
EC REP	Authorized Representative in the European Community		Date of Manufacture
	Biological risks		Caution
	Do Not Freeze		Do not reuse
Made in USA of US and Foreign Components		Made in USA of US and Foreign Components	

REFERENCES

1. National Institute on Drug Abuse Research, "Urine Testing for Drugs of Abuse", Monograph 73 (1986).
2. National Institute on Drug Abuse, "Mandatory Guidelines for Federal Workplace Drug Testing Programs", *Federal Register*, Vol. 53, No. 69 (1988).
3. Rubenstein, K. E., Schneider, R. S., Ullman, E. F., 'Homogenous Enzyme Immunoassay: A New Immunochemical Technique", *Biochem. Biophys. Res. Commun.* , Vol. 47, 846 851 (1972).
4. McEnvoy, G. K., Litvak, K., *AHFS Drug Information*, American Society of Hospital Pharmacists, Inc., Bethesda, MD (1992).
5. Wyngarrden, J. B., Smith, L. H. Jr., Cecil, *Textbook of Medicine*, W. B. Saunders Company, Philadelphia, PA (1982).
6. National Committee for Clinical Laboratory Standards, *Urine Drug Testing in the Clinical Laboratory*, Proposed Guideline, Vol.13, No. 7, NCCLS publication T/DM8-P, Villanova, PA (1993).
7. "USP XXII, NF XVII", United States Pharmacopeial Convention, Inc., Rockville, MD (1990).
8. CDC-NIH, *Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition, (Washington, D.C.: U.S. Government Printing Office, 2009). (CDC 21-1112)
9. Substance Abuse and Mental Health Service Administration, "Mandatory Guidelines for Federal Workplace Drug Testing Programs", *Federal Register*, Vol. 58, No. 14, (1993).
10. Crane, T., et al., "Mefenamic Acid Prevents Assessment of Drug Abuse with EMIT™ Assays", *Clin. Chem.*, Vol. 39, No. 3, 549 (1993).
11. Tietz, N. W., *Fundamentals of Clinical Chemistry*, 4th Edition, W. B. Saunders Company, Philadelphia, PA (1996).

EC REP Beckman Coulter Eurocenter S.A., 22, rue Juste-Olivier. Case Postale 1044, CH - 1260 Nyon 1, Switzerland
Tel: +41 (0)22 365 36 11

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
www.beckmancoulter.com
Manufactured for Beckman Coulter, Inc.