Acetaminophen (ACTM) Technical Procedure 3100T
Beckman UniCel DxC Systems

Principle

Intended Use

ACTM reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Drug Calibrator 2 set, is intended for quantitative determination of acetaminophen concentration in human serum.

Clinical Significance

Acetaminophen (paracetamol) is a common drug which is used as an analgesic and an antipyretic agent. Excessive doses of acetaminophen can have toxic effects with the most common being hepatotoxicity. The drug may also cause acute tubular necrosis, pancreatitis, and myocardial necrosis.(1) Diagnosis of acetaminophen overdose can be determined by measuring the circulating levels of acetaminophen in order that treatment can be initiated.(2)

Methodology

ACTM reagent is used to measure analyte concentration by a particle enhanced turbidimetric inhibition immunoassay method.(3) Particle-bound drug (PBD) binds to the Acetaminophen specific antibody (Ab) resulting in the formation of insoluble aggregates causing light scatter. Non-particle-bound Acetaminophen in the patient sample competes with the PBD for the antibody binding sites, inhibiting the formation of insoluble aggregates. The rate and amount of particle aggregation is inversely proportional to the concentration of Acetaminophen in the sample.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 60 parts reagent. The system monitors the aggregate formation by measuring the change in absorbance at 340 nanometers. This change in absorbance is inversely proportional to the concentration of ACTM in the sample and is used by the System to calculate and express the ACTM concentration based upon a multi-point calibration curve.

Chemical Reaction Scheme

Acetaminophen (sample) + PBD ~~+ Ab~~ → PBD-Ab (Aggregates) + Acetaminophen (sample)-Ab

Specimen

Acceptable Sample Containers

13 x 75 Red Top BD tubes

Red Top BD microtainers

13 x 75 Sodium Heparin BD tubes

Sodium Heparin microtainers

Optimum volume: 0.5 mL, Minimum volume: 0.1 mL

Unacceptable Sample Containers

Whole blood or urine and SST/PST samples are not recommended for use as a sample.

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(4) Freshly drawn serum is the preferred specimen. Serial samples should be collected using the same sample type (i.e., serum or plasma).

Specimen Storage and Stability

Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.(5)

Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C.

Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.(5)

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 UniCel DxC Systems.

Criteria for Unacceptable Specimens

Refer to the *Procedural Notes* section of this procedure for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items:

One ACTM Reagent Cartridge (1 x 100 tests) Kit Reorder #472169

Volumes per Test

Sample Volume	5 µL
Total Reagent Volume	302 µL

Cartridge Volumes

A	230 µL
B	40 µL
C	32 µL

Reactive Ingredients

Reagent Constituents

Acetaminophen Particle Reagent	4.8 mL
Monoclonal anti-Acetaminophen Antibody (mouse)	7.0 mL
Acetaminophen Reaction Buffer	80.0 mL

Also non-reactive chemicals necessary for optimal system performance

CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

Materials Needed but Not Supplied With Reagent Kit

SYNCHRON and UniCel DxC Systems Drug Calibrator 2 set

At least two levels of control material

Saline

Reagent Preparation

No preparation is required. Do not mix.
Document lot number in reagent log, date and initial every cartridge before loading.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

Reagent Storage and Stability

ACTM 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 42 days at 2°C to 8°C unless the expiration date is exceeded. **DO NOT FREEZE.** Do not expose reagent to temperatures above 35°C or to direct sunlight.

Equipment

This test is performed on the Beckman UniCel DxC 800 Systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the Beckman *UniCel DxC 800 systems Reference Manual* for detailed instructions.

Calibration

Calibrator Required

SYNCHRON® Systems Drug Calibrator 2 set (6 point calibration) **Kit Reorder #469630**

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

SYNCHRON® Systems Drug Calibrator 2 set is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at 2°C to 8°C.

CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.6

Calibration Information

1. The system must have a valid calibration curve in memory before control or patient samples can be run.
2. Under typical operating conditions the ACTM reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC800 System *Instructions For Use (IFU)* manual. This assay has within-lot calibration available. Refer to the UniCel DxC800 System *Instructions For Use* (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the UniCel DxC800 System *Instructions For Use* (IFU) manual.
4. 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

The measurand (ACTM) in this calibrator is traceable to the Manufacturer's Working Calibrator. The traceability process is based on prEN ISO 17511.

The set point values were established based upon the gravimetric addition of specific quantities of the measurand to achieve the appropriate concentration. The values were verified using representative samples from this lot of calibrator are specific to the assay methodologies of the SYNCHRON reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

SYNCHRON® Systems Drug Calibrator 2 is prepared using processed human serum to which weighed-in drug

quantities are added,

Quality Control

At least two levels of control material should be analyzed each shift. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxC800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the *Control IFUs* folder on the S drive (*S:\APS\ClinLab\PoliciesandProcedures\1000- 8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\Control IFUs*). Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage
MAS ChemTrak 1	+2°C to +8°C
MAS ChemTrak 3	+2°C to +8°C

Controls are received frozen and stored at –10°C to –20°C.
Bottles of controls in use are thawed and stored at 2°C to 8°°C and are good for 14 days.

Testing Procedure

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 operation.

For detailed testing procedures, refer to the UniCel DxC800 System *Instructions For Use* (IFU) manual.

Calculations

UniCel DxC Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

Reporting Results

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

Reference Intervals

In cases of suspected overdose, determination of serum acetaminophen concentration 4 or more hours after ingestion is recommended to identify potential hepatotoxicity. When the type of acetaminophen ingested is not known or includes an extended release product, a second acetaminophen level should be obtained 4 to 6 hours after the first level is measured.(8)

Critical Value: Acetaminophen results **> 120 mg/L** are critical and should immediately be called to the patient's nurse or physician.

Reference Intervals

Intervals	Sample Type	Conventional Units	S.I. Units
Therapeutic	Serum/Plasma	10-30 mg/L	66-199 umol/L
Hepatotoxic	Serum/Plasma 4 hours post-ingestion	> 150 mg/L	> 993 umol/L
	Serum/Plasma 8 hours post ingestion	> 75 mg/L	> 496 umol/L
	Serum/Plasma 12 hours post-ingestion	> 40 mg/L	> 265 umol/L

Refer to References (9,10,11) for guidelines on establishing laboratory-specific reference intervals.

Procedural Notes

Anticoagulant Test Results

The following anticoagulants were assessed by Deming regression analysis with a minimum of 50 paired serum and plasma samples. Values of serum (X) ranging from 23 to 280 µg/mL were compared with the values from plasma (Y) yielding the following results:

Acceptable Anticoagulants (**Non-Gel Tubes ONLY**)

Anticoagulant	Level of Anticoagulant Tested	Deming Regression Analysis
Lithium Heparin	14 Units/mL	Y = 1.037X + 3.9; r = 0.994
Sodium Heparin	14 Units/mL	Y = 1.022X + 5.2; r = 0.996

Limitations

Samples reported out as "SUPPRESSED" due to RXN ERROR should be reanalyzed.

Interferences

The following substances were tested for interferences with this methodology:

Substance	Source	Level	Observed Effect
Hemoglobin	RBC Hemolysate	500 mg/dL	NSI[a]
Bilirubin	Porcine	30 mg/dL	NSI
Rheumatoid Factor	Human	300 IU/mL	NSI
Lipemia	Human	4+	NSI
Paraprotein	Human	500 mg/dL	NSI

[a] NSI = No Significant Interference (within ± 4 mg/L or 8%).

Refer to References (12,13,14) for other interferences caused by drugs, disease and preanalytical variables.

For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Human anti-mouse antibodies may be present in samples from patients who have received immunotherapy or diagnostic procedures utilizing monoclonal antibodies or in individuals who have been regularly exposed to animals.(15,16) Additionally, other heterophile antibodies, such as human anti-goat antibodies may be present in patient samples. Interpretation of results should be done in the context of the overall clinical presentation of the patient, including symptoms, clinical history, data from additional tests and other appropriate information.

Specificity

The following list of substances were added at the concentration listed to separate aliquots of a serum pool containing 40 µg/mL of the analyte. In most cases the value shown approximates maximum physiological concentrations. The recovered values were subtracted from the serum pool value. If the recovered results were within two times the within-run precision specifications there was no significant interference. If the recovered results were more than two times the within-run precision specifications the difference is listed under observed effect.

Specificity[a]

Substance	Concentration (µg/mL)	Observed Effect
Acetaminophen Glucuronide	1000	NSI[b]
Acetaminophen Sulfate	1000	NSI
Acetophenetidin	1000	NSI
N-Acetyl-L-cysteine	1000	NSI
Amitriptyline	1000	NSI
Caffeine	1000	NSI
Cimetidine	1000	NSI
Codeine	1000	NSI
D-Cysteine, HCL	1000	NSI
DL-Cysteine, HCL	1000	NSI
L-Cysteine, HCL	1000	NSI
3-Cysteine Acetaminophen	1000	NSI
Diazepam	1000	NSI
Mercapturic Acetaminophen	900	NSI
D-Methionine	1000	NSI
DL-Methionine	1000	NSI
L-Methionine	1000	NSI
Nicotine	50	NSI
Penicillin V	1050	NSI
Phenobarbital	525	NSI

L-Phenylephedrine	1000	NSI
Primidone	50	NSI
D-Propoxyphene, HCL	1000	NSI
Procainamide	800	NSI
Secobarbital	1000	NSI
Sodium Salicylate	1000	NSI

[a] Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

[b] NSI = No Significant Interference (within ± 8%).

Performance Characteristics

Analytical Measurement Range

The UniCel DxC System(s) method for the determination of this analyte provides the following analytical ranges:

Analytical Measurement Range (AMR)

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	10 – 300 mg/L	66 – 1986 umol/L

Clinical Reportable Range

Clinical Reportable Range (CRR) as determined at UCDMC

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	10 – 400 mg/L	66 – 2648 umol/L

The analytical reportable range of this assay is 10 - 300 mg/L. A low ACTM result is set to “print” down to the low reportable limit of 0.1 mg/L, which is below the analytical limit (**10 mg/L**),. All samples with results below (Less than or suppressed OIR low) 10 mg/L will be reported as "**< 10 mg/L**" by the Remisol Data Manager.

Samples with concentrations greater than the AMR **(> 300 mg/L**) should be diluted (X2) with saline and re-analyzed. Results are reported up to the Clinical Reportable Range (400 mg/L). Results from dilutions that exceed the CRR are be reported as "**> 400 mg/L**" unless the diluted result is requested by the physician.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

Samples reported out as "SUPPRESSED" due to RXN ERROR should be reanalyzed.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for ACTM determination is 2.5 mg/L (16 µmol/L).

Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

As determined by Beckman

Serum or plasma (in the range of 1.0 to 278 µg/mL):

Y (SYNCHRON LX Systems)	= 0.988X + 4.0
N	= 131
MEAN (SYNCHRON LX Systems)	= 91.8
MEAN (Fluorescence Polarization Immunoassay)	= 88.8 Correlation Coefficient (r) = 0.992

Refer to References (17) for guidelines on performing equivalency testing.

As determined at UCDMC

Serum (in the range of 7.2 to 140.6 mg/L):

Y (DxC800-1805)	= 1.011X + 1.97 mg/L
N	= 22
MEAN (DxC800-1805)	= 48.66 mg/L
MEAN (DxC800-4118)	= 51.17 mg/L
CORRELATION COEFFICIENT (r)	= 0.9990

Serum (in the range of 7.2 to 140.6 mg/L):

Y (UniCel DxC800-1805)	= 1.047X + 0.87
N	= 22
MEAN (UniCel DxC800-1805)	= 48.66
MEAN (UniCel DxC800-4427)	= 51.82
CORRELATION COEFFICIENT (r)	= 0.9988

Serum (in the range of 7.2 to 140.6 mg/L):

Y (UniCel DxC800-1805)	= 1.004X + 1.83
N	= 22
MEAN (UniCel DxC800-1805)	= 48.66
MEAN (UniCel DxC800-4449)	= 50.66
CORRELATION COEFFICIENT (r)	= 0.9987

Serum (in the range of 8.7 to 144.6 mg/L):

Y (UniCel DxC800-4118)	= 1.035X - 1.16
N	= 22
MEAN (UniCel DxC800-4118)	= 51.17
MEAN (UniCel DxC800-4427)	= 51.82
CORRELATION COEFFICIENT (r)	= 0.9989

Serum (in the range of 8.7 to 144.6 mg/L):

Y (UniCel DxC800-4118)	= 0.992X - 0.13
N	= 22
MEAN (UniCel DxC800-4118)	= 51.17
MEAN (UniCel DxC800-4449)	= 50.66
CORRELATION COEFFICIENT (r)	= 0.9990

Serum (in the range of 9.6 to 149.6 mg/L):

Y (UniCel DxC800-4427)	= 0.959X + 0.99
N	= 22
MEAN (UniCel DxC800-4427)	= 51.82
MEAN (UniCel DxC800-4449)	= 50.66
CORRELATION COEFFICIENT (r)	= 0.9994

Precision

A properly operating UniCel DxC System(s) should exhibit precision values less than or equal to the following:

As determined by Beckman

Precision Values

Type of Precision	Sample Type	1 SD		Changeover Value[a]		%CV
		ug/mL	umol/L	ug/mL	umol/L	
Within-run	Serum/Plasma	2.0	13.2	50.0	33.0	4.0
Total	Serum/Plasma	3.0	19.9	50.0	33.0	6.0

[a] When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Precision established at UCDMC

DxC800-4118

Type of Precision	Sample Type	n	Mean (mg/L)	1 SD	%CV
DxC800-4118 Within-run	Beckman TDM 1	20	19.94	0.97	4.9
	Beckman TDM 3	20	146.69	2.61	1.8
DxC800-4427 Within-run	Beckman TDM 1	20	20.60	1.24	6.0
	Beckman TDM 3	20	141.11	2.99	2.1
DxC800-4449 Within-run	Beckman TDM 1	20	19.64	0.97	4.9
	Beckman TDM 3	20	139.12	3.36	2.4

Type of Imprecision	Sample Type	n	Mean (mg/L)	SD	%CV
DxC800-4118 Day to Day	MAS ChemTrak 1	18	27.6	1.65	6.0
	MAS ChemTrak 3	18	136.6	4.07	3.0
DxC800-4427 Day to Day	MAS ChemTrak 1	367	27.4	1.65	6.0
	MAS ChemTrak 3	366	134.2	4.84	3.6
DxC800-4449 Day to Day	MAS ChemTrak 1	350	27.2	1.63	6.0
	MAS ChemTrak 3	349	135.3	4.42	3.3

Comparative performance data for the SYNCHRON LX® System evaluated using the NCCLS Approved Guideline EP5-A appears in the following table.(18)

Comparative performance as determined by Beckman

NCCLS EP5-A Precision Estimate Method

Type of Imprecision	Sample Type		No. Systems	No. Data Points[a]	Test Mean Value (ug/mL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	20	0.9	4.3
	Serum	Control 2	1	80	72	1.7	2.4
	Serum	Control 3	1	80	142	3.0	2.1
Total	Serum	Control 1	1	80	20	2.0	10.0
	Serum	Control 2	1	80	72	3.3	4.6
	Serum	Control 3	1	80	142	6.2	4.4

[a] The point estimate is based on the data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on the SYNCHRON LX and UniCel DxC Systems 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.

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	March, 2003	AxSym #5210.T

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
			04/16/2003	S. Devaraj
			10/25/2004	S. Devaraj
			12/15/2005	S. Devaraj
			10/10/2006	S. Devaraj
			11/05/2007	G. Kost
11/13/2008	Lower limit dilution procedure	M. Inn		
			06/16/2008	G. Kost
			09/15/2009	G. Kost
02/28/2010	Lower Limit Dilution Procedure removed-software update	M. Inn		
			10/12/2010	G. Kost
01/19/2011	Acceptable sample type update	M. Inn		
06/13/2011	General update	M.Inn	11/16/2011	G. Kost
			09/17/2013	G. Kost
03/20/2014	Added Sodium Heparin sample type to acceptable samples	kdagang	04/15/2015	J. Gregg