Tobramycin (TOB) - Serum
Beckman UniCel DxC Systems

Technical Procedure 3154

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Tobramycin (TOB) - Serum
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For *In Vitro* Diagnostic Use Only

Principle

Intended Use

TOB reagent, when used in conjunction with UniCel® DxC 800 Systems and SYNCHRON® Systems Drug Calibrator 3 Plus set, is intended for quantitative determination of tobramycin concentration in human serum.

Clinical Significance

Tobramycin is an antibiotic and is monitored for effectiveness of the dose and possible nephrotoxicity.

Methodology

TOB reagent is used to measure tobramycin concentration by a particle enhanced turbidimetric inhibition immunoassay method.(1) Particle-bound drug (PBD) binds Tobramycin specific antibody (Ab) resulting in the formation of insoluble aggregates causing light scatter. Non-particle-bound tobramycin 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 tobramycin 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 90 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 TOB in the sample and is used by the System to calculate and express the TOB concentration based upon a multi-point calibration curve.

Chemical Reaction Scheme

Tobramycin (sample) + PBD + Ab ⟶ PBD-Ab (Aggregates) + Tobramycin (sample)-Ab

Acceptable Sample Containers

13 x 75 Red Top BD tubes
Red Top BD microtainers
Optimum volume: 0.5 mL, Minimum volume: 0.1 mL

Specimen

Type of Specimen

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.(2) Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in the *Procedural Notes* section of this chemistry information sheet. Serial samples should be collected using the same sample type (i.e., serum or plasma). **The default sample type is serum.**

Specimen Storage and Stability

1. 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.(3)
2. 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.(3)

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 or the *Specimen Requirements* section of this procedure for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items:
Two TOB Reagent Cartridges (2 x 100 tests) **Kit Reorder #467983**

Volumes per Test

Sample Volume	3 µL
Total Reagent Volume	270 µL

Cartridge Volumes

A	200 µL
B	40 µL
C	30 µL

Reactive Ingredients

Reagent Constituents	
Tobramycin Particle Reagent	6.5 mL
Monoclonal anti-Tobramycin Antibody (mouse)	4.8 mL
Tobramycin Reaction Buffer	100.0 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 3 Plus set
At least two levels of control material
Saline

Reagent Preparation

No preparation is required. Do not mix.
Date and initial cartridge and document in reagent log before loading each new cartridge.

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 *Quality Control* section of this procedure.

Reagent Storage and Stability

TOB 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 3 Plus set (6 point calibration) **Kit Reorder #471080**

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

SYNCHRON® Systems Drug Calibrator 3 Plus 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.(4)

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 TOB 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 (TOB) 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 3 Plus 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 or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Copies of these inserts can be found in the *Control Procedures* section of the Beckman UniCel DxC *Chemistry Information Manual*. 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.

Table 1 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

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

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

C. Program samples and controls for analysis.

D. 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.

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.

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

Therapeutic TOB concentrations vary significantly, depending upon the individual. The lower limit for one patient may be ineffective in another, while the upper limit may prove toxic in a third. The physician should determine the appropriate reference interval for each patient. The reference intervals listed below were taken from the literature.(5)

Table 2 Reference intervals

Intervals	Sample Type	Drug Level	Therapeutic Interval		Toxic Interval	
			Conventional Units (µg/mL)	S.I Units (µmol/L)	Conventional Units (µg/mL)	S.I Units (µmol/L)
Literature	Serum or Plasma	Peak			> 10 - 12	> 21 - 26
		Less severe infection	5 - 8	11 - 17		
		Severe Infection	8 - 10	17 - 21		
		Trough			> 2 - 4	> 4 - 9
		Less severe infection	< 1	< 2		
		Moderate Infection	< 2	< 4		
		Severe Infection	< 2 - 4	< 4 - 9		
UCDMC*	**Serum**	**Peak**	**5.0 - 10.0**	**10.7 - 21.4**		
		Trough	**≤ 2.0**	**≤ 4.3**		

Refer to References (6,7,8) for guidelines on establishing laboratory-specific reference intervals.

*The following comment will be attached to each result:

The concentrations refer to conventional multiple-daily dosing. The target peak concentration depends upon the severity and type of infection being treated. Prolonged exposure to trough levels >2.0 ug/mL and/or peak levels >12.0 ug/mL may lead to toxicity.

Procedural Notes

Anticoagulant Test Results

1. If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Table 3 Acceptable Anticoagulants[a] **These sample types are for non-gel tubes only.**

Anticoagulant	Level Tested for In vitro Interference	Average Plasma-Serum Bias (µg/mL)
Lithium Heparin	14 Units/mL	NSI[b]
Sodium Heparin	14 Units/mL	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 ± 0.4 µg/mL or 8%).

Limitations

None identified.

Interferences

1. The following substances were tested by Beckman Coulter for interference with this methodology:

Table 4 Interferences[a]

Substance	Source	Level	Observed Effect
Hemoglobin	RBC Hemolysate	500 mg/dL	NSI[b]
Bilirubin	Porcine	30 mg/dL	NSI
Rheumatoid Factor	Human	300 IU/mL	NSI
Lipemia	Human	3+	NSI
Paraprotein (IgM)	Human	500 mg/dL	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.

[c] NSI = No Significant Interference (within ±0.4 µg/mL or 8%)

2. Refer to References (9,10,11) for other interferences caused by drugs, disease and preanalytical variables.
3. 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.(12,13) 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

Peak Tobramycin Level

The following list of substances were added at the concentration listed to separate aliquots of a serum pool containing 6.1 µg/mL tobramycin. In most cases the value shown approximates maximum physiological concentrations. The recovered values were subtracted from the serum pool value. If the results were within ± 2X of the within-run precision specifications there was no significant interference. If the recovered results were more than ± 2X of the within-run precision specifications the difference is listed under observed effect.

Table 5 Specificity - Peak Tobramycin Level[a]

Substance	Concentration (µg/mL)	Observed Recovery (µg/mL)	Observed Effect (µg/mL)
Amikacin	70	6.2	NSI[b]
Ampicillin	100	5.7	NSI
Carbenicillin	250	5.8	NSI
Cephalothin	500	6.9	+13%
Chloramphenicol	50	6.5	NSI
Clindamycin	100	6.2	NSI
Erythromycin	40	6.2	NSI
Gentamicin	20	6.0	NSI
Kanamycin A	1.4	6.4	NSI
Lincomycin	100	6.9	+13%
Neomycin	60	6.5	NSI
Netilmycin	20	6.3	NSI
Penicillin	50	6.3	NSI
Sisomicin	10	6.1	NSI
Streptomycin	60	6.4	NSI
Sulfanilimide	100	6.4	NSI
Tetracycline	500	6.9	+13%
Trimethoprin	500	7.4	+21%
Vancomycin	100	6.5	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%).

Trough Tobramycin Level

The following list of substances were added at the concentration listed to separate aliquots of a serum pool containing 2.7 µg/mL tobramycin. In most cases the value shown approximates maximum physiological concentrations. The recovered values were subtracted from the serum pool value. If the results were within ± 2X of the within-run precision specifications there was no significant interference. If the recovered results were more than ± 2X of the within-run precision specifications the difference is listed under observed effect.

Table 6 Specificity - Trough Tobramycin Level[a]

Substance	Concentration (µg/mL)	Observed Recovery (µg/mL)	Observed Effect (µg/mL)
Amikacin	70	2.7	NSI[b]
Ampicillin	100	2.7	NSI
Carbenicillin	250	2.7	NSI
Cephalothin	500	2.7	NSI
Chloramphenicol	50	2.7	NSI
Clindamycin	100	2.7	NSI
Erythromycin	40	2.6	NSI
Gentamicin	20	2.7	NSI
Kanamycin A	1.4	4.3	+1.6
Lincomycin	100	2.7	NSI
Neomycin	60	2.7	NSI
Netilmycin	20	2.7	NSI
Penicillin	50	2.7	NSI
Sisomicin	10	2.8	NSI
Streptomycin	60	2.7	NSI
Sulfanilimide	100	2.7	NSI
Tetracycline	500	2.8	NSI
Trimethoprin	500	2.8	NSI
Vancomycin	100	2.7	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 ± 0.4 µg/mL).

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Performance Characteristics

Analytical Measurement Range

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

Table 7 Analytical Measurement Range (AMR)

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	0.5 - 12.0 µg/mL	1.1 - 25.7 µmol/L

Clinical Reportable Range

Table 8 Clinical Reportable Range (CRR) as determined at UCDMC

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	0.5 - 40.0 µg/mL	1.1 - 85.7 µmol/L

Samples with concentrations less than the AMR and CRR will be reported as "**< 0.5 µg/mL**" ("< 1.1 µmol/L"). Samples with concentrations greater than the AMR will be diluted with saline and diluted results greater than the CRR will be reported as "**> 40.0 µg/mL**" ("> 85.7 µmol/L").

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.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for TOB determination is 0.5 µg/mL (1.1 µmol/L).

Equivalency

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

Equivalency determined by Beckman Coulter:

Serum or plasma (in the range of 0.6 to 11.4 µg/mL):

Y (SYNCHRON LX Systems)	= 1.009X + 0.04
N	= 69
MEAN (SYNCHRON LX Systems)	= 3.38
MEAN (SYNCHRON CX4CE)	= 3.32
Correlation Coefficient (r)	= 0.9980

Equivalency determined at UCDMC:

Serum (in the range of 0.4 to 11.6 µg/mL):

Y (DxC800-4427)	= 1.1611X - 0.136
N	= 43
MEAN (DxC800-4427)	= 4.1
MEAN (Abbott AxSym)	= 3.7
CORRELATION COEFFICIENT (r)	= 0.9907

Serum (in the range of 0.4 to 11.6 µg/mL):

Y (UniCel DxC800-4449)	= 1.1310X - 0.054
N	= 43
MEAN (UniCel DxC800-4449)	= 4.1
MEAN (Abbott AxSym)	= 3.7
CORRELATION COEFFICIENT (r)	= 0.9908

Serum (in the range of 0.2 to 12.0 µg/mL):

Y (UniCel DxC800-4449)	= 0.9742X + 0.078
N	= 43
MEAN (UniCel DxC800-4449)	= 4.1
MEAN (UniCel DxC800-4427)	= 4.1
CORRELATION COEFFICIENT (r)	= 0.9970

Refer to References (14) for guidelines on performing equivalency testing.

Precision

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

Table 9 Precision Values

Type of Precision	Sample Type	1 SD		Changeover Value[a]		%CV
		µg/mL	µmol/L	µg/mL	µmol/L	
Within-run	Serum/Plasma	0.2	0.4	5.0	10.0	4.0
Total	Serum/Plasma	0.3	0.6	5.0	10.0	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

DxC800-4427

Type of Precision	Sample Type	Mean (µg/mL)	1 SD	%CV
Within-run	MAS ChemTrak 1	2.0	0.10	4.8
	MAS ChemTrak 3	8.5	0.12	1.4

DxC800-4449

Type of Precision	Sample Type	Mean (µg/mL)	1 SD	%CV
Within-run	MAS ChemTrak 1	2.1	0.07	3.5
	MAS ChemTrak 3	8.6	0.11	1.3

Comparative performance data for the SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.(15)

Table 10 NCCLS EP5-T2 Precision Estimate Method

Type of Imprecision	Sample Type	No. Systems	No. Data Points[a]	Test Mean Value (µg/mL)	EP5-T2 Calculated Point Estimates	
					SD	%CV
Within-run	Serum Control 1	1	80	1.5	0.04	3.0
	Serum Control 2	1	80	6.0	0.09	1.6
	Serum Control 3	1	80	10.9	0.16	1.5
Total	Serum Control 1	1	80	1.5	0.06	4.4
	Serum Control 2	1	80	6.0	0.13	2.1
	Serum Control 3	1	80	10.9	0.18	1.7

[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.

Day to Day Precision established at UCDMC

DxC800-4427

Type of Imprecision	Sample Type	No. Systems	No. Data Points	Test Mean Value (µg/mL)	SD	%CV
Day to Day	MAS ChemTrak 1	1	59	1.9	0.16	8.4
	MAS ChemTrak 3	1	56	8.2	0.30	3.7

DxC800-4449

Type of Imprecision	Sample Type	No. Systems	No. Data Points	Test Mean Value (µg/mL)	SD	%CV
Day to Day	MAS ChemTrak 1	1	144	2.0	0.15	7.5
	MAS ChemTrak 3	1	144	8.2	0.24	2.9

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 UniCel DxC800 System *Instructions For Use* (IFU) manual.

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