

Principle

Intended Use

VANC reagent, when used in conjunction with UniCel® DxC 800 Systems and SYNCHRON® Systems Vancomycin Calibrator set, is intended for quantitative determination of vancomycin concentration in human serum or plasma.

Clinical Significance

Vancomycin is a glycopeptide antibiotic. It is primarily used in the treatment of infections due to β -lactam or methicillin-resistant gram positive cocci and metronidazole-resistant gram positive bacilli.(1) It may also be used prophylactically in some patients who are at risk for endocarditis or when methicillin-resistant *Staphylococcus aureus* or *Staphylococcus epidermidis* is a risk. (2) Since individual patients exhibit a high degree of variability in absorption and metabolism of vancomycin, therapeutic monitoring is recommended in certain patients. (1) The major toxic reactions include “red-man syndrome” nephrotoxicity, and ototoxicity when very high levels of the drug are present in the circulation.

Methodology

VANC reagent is used to measure the vancomycin concentration by a particle enhanced turbidimetric inhibition immunoassay method.(3) Particle-bound drug (PBD) binds to vancomycin specific antibody (Ab) resulting in the formation of insoluble aggregates causing light scatter. Non-particle-bound vancomycin 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 upon the concentration of vancomycin 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 104 parts reagent. The system monitors aggregate formation by measuring the change in absorbance at 340 nanometers. This change in absorbance is inversely proportional to the concentration of vancomycin in the sample and is used by the System to calculate and express the vancomycin concentration based upon a multi-point calibration curve.

Chemical Reaction Scheme



Specimen

Acceptable Sample Containers

- 13 x 75 Sodium Heparin BD tubes
- Sodium Heparin microtainers
- 13 x 75 Red Top BD tubes
- Red Top BD microtainers

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 or plasma are the preferred specimens. 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:

Two VANC Reagent Cartridges (2 x 100 tests) [Kit Reorder #474824](#)

Volumes per Test

Sample Volume	3 µL
ORDAC Sample Volume	2 µL
Total Reagent Volume	312 µL

Cartridge Volumes

A	230 µL
B	50 µL
C	32 µL

Reactive Ingredients

Reagent Constituents

Vancomycin Particle Reagent	8 mL
Monoclonal anti-vancomycin Antibody (mouse)	5 mL
Vancomycin Reaction Buffer	55 mL

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 Hazards (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.

Materials Needed but Not Supplied With Reagent Kit

- SYNCHRON Systems Vancomycin Calibrator set
- At least two levels of control material
- Saline

Reagent Preparation

Gently invert the cartridge three times prior to loading onto the SYNCHRON System.

FAILURE TO MIX THE REAGENT WILL RESULT IN ERRONEOUS VALUES.

Check for bubbles and/or foam in the compartments; break any bubbles.

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

VANC 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 Vancomycin Calibrator set (6 point calibration) [Kit Reorder 378045](#)

Calibrator Preparation

No preparation is required. Mix contents with a gentle swirling motion and transfer the calibration solution into the sample cups. Recap calibrator bottles tightly and refrigerate when not in use. Do not mix caps among the various bottles in the calibrator set.

Calibrator Storage and Stability

SYNCHRON® Systems Vancomycin Calibrator 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. Do not freeze.

Calibration Information

The system must have a valid calibration curve in memory before control or patient samples can be run.

Under typical operating conditions the VANC 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.

For detailed calibration instructions, refer to the UniCel DxC800 System [Instructions For Use \(IFU\)](#) manual.

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 (VANC) in this calibrator is traceable to the Manufacturer's Working Calibrator. The Working Calibrator is prepared by diluting the concentrate gravimetrically. The concentrate is prepared from USP grade Vancomycin₂. The traceability process is based on prEN ISO 17511.

The values were verified using representative samples from this lot of Calibrator and are specific to the assay methodologies of the SYNCHRON systems. Values determined by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

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. 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.

Reporting Results

Reference Intervals

The reference intervals listed below were taken from the literature. (1,2,6,7)

Reference Intervals

Intervals	Sample Type	Conventional Units
Therapeutic		
Trough	Serum/Plasma	5 – 15 µg/mL
Peak	Serum/Plasma	20 – 40 µg/mL
Toxic	Serum/Plasma	> 40 µg/mL (6)

Refer to References (8,9,10) 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 4.2 to 49.0 µg/mL were compared with the values from plasma (Y) yielding the following results:

Acceptable Anticoagulants These samples types are for non-gel tubes only.

Anticoagulant	Level of Anticoagulant Tested	Deming Regression Analysis
Lithium Heparin	14 Units/mL	$Y = 1.000X - 0.03; r = 0.997$
Sodium Heparin	14 Units/mL	$Y = 0.990X - 0.19; r = 0.998$

Limitations

None identified.

Interferences

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

Interferences

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 (IgM)	Human	500 mg/dL	NSI

^a NSI = No Significant Interference (within ± 2.6 µg/mL or 8%)

Interference may occur with serum samples from patients diagnosed as having plasma cell dyscrasias and lymphoreticular malignancies associated with abnormal immunoglobulin synthesis, such as multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease. (11) Results for these samples are typically suppressed. If using the dilution protocol for results less than the analytical range, results from patients with these disease states usually do not approximate the known value.

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.

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

Specificity

The substances in the following table were added at the concentration listed to separate aliquots of a serum pool containing 15 µg/mL vancomycin. 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 ± 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.

Specificity^a

Substance	Concentration (µg/mL)	Observed Effect (mg/L)
Vancomycin Crystalline Degradation Product (CDP-1)	320	NSI ^b
Acetaminophen	1000	NSI
Amikacin	500	NSI
Amphotericin B	138	NSI
Caffeine	500	NSI
Carbenicillin	525	NSI
Cefazolin	490	NSI
Chloramphenicol	510	NSI
Clindamycin	525	NSI
Erythromycin	490	NSI
Furosemide	520	NSI
Gentamicin	625	NSI
Heparin	600	NSI
Ibuprofen	675	NSI
Kanamycin A	550	NSI
Kanamycin B	550	NSI
Lincomycin	550	NSI
Methicillin	505	NSI
Methotrexate	236	NSI
Neomycin	500	NSI
Netilmicin	500	NSI
Penicillin G	625	NSI
Phenacetin	515	NSI

Prednisolone	340	NSI
Refampin	290	NSI
Salicylic acid	775	NSI
Sulfamethoxazole	500	NSI
Tetracycline	500	NSI
Tobramycin	525	NSI
Trimethoprim	505	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 ($\pm 2.6 \mu\text{g/mL}$).

Performance Characteristics

Analytical Measurement Range

The UniCel DxC System(s) method for the determination of vancomycin provides the following analytical range.

Analytical Measurement Range (AMR)

Sample Type	Range ($\mu\text{g/mL}$)
Serum or Plasma	3.5 – 40 $\mu\text{g/mL}$
Serum or Plasma (ORDAC)	30 – 60 $\mu\text{g/mL}$

Clinical Reportable Range

Clinical Reportable Range (CRR) as determined at UCDCM

Sample Type	Range ($\mu\text{g/mL}$)
Serum or Plasma	3.5 – 100 $\mu\text{g/mL}$

Samples with concentrations greater than the AMR ($> 40 \mu\text{g/mL}$) will be rerun using the ORDAC function.

Samples with concentrations $> 60 \mu\text{g/mL}$ are to be diluted 1:2 with saline and reanalyzed. Diluted results can be reported out up to the Clinical Reportable Range (100 $\mu\text{g/mL}$).

Results from dilution exceeding the CRR are to be reported as " **$> 100 \mu\text{g/mL}$** ".

The analytical reportable range of this assay is 3.5 – 100 $\mu\text{g/mL}$. Low VANC results from the instrument are set to "print" below the analytical limit down to the lowest reportable limit of 0.1 $\mu\text{g/mL}$. All samples with results **below 0.1 $\mu\text{g/mL}$ ("less than" or "suppressed OIR low")** will need to be confirmed by dilution. (The messages window on the Remisol Data Manager screen will request that you make an offline dilution with ChemTrak 3) Printed results between 0.1 $\mu\text{g/mL}$ and 3.4 $\mu\text{g/mL}$ do not need to be confirmed by dilution and are reported as " **$< 3.5 \mu\text{g/mL}$** ".

Dilution protocol: Confirm a suspected low VANC sample result by adding one measured volume of test sample to an equal volume of a sample with known VANC concentration (MAS ChemTrak 3). The assayed VANC result multiplied by 2 of this diluted sample should be within $\pm 2\text{SD}$ of MAS ChemTrak 3 VANC mean. If the multiplied assayed result of the diluted sample is not within the acceptable control range, the VANC sample should be reported out as "**Unable to determine due to unknown interferences.**" (canned text **GINT**).

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

The following comments will be automatically appended to VANC results by LIS:

Vancomycin (Random), (canned text **VANCORAN2**):

Actual recommended therapeutic levels depend upon the treatment regimen for the patient.

Random Vancomycin values can have a large range of values.

The Therapeutic Reference interval for PEAK values is 20.0 – 40.0 ug/mL and TROUGH values is 5.0 – 15.0 ug/mL.

Vancomycin (Peak) and/or Vancomycin (Trough), (canned text **VANTREAT**):

Actual recommended therapeutic levels depend upon the treatment regimen for the patient.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for VANC determination is 3.5 µg/mL.

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 3.6 to 51.6 µg/mL):

Y (SYNCHRON LX Systems)	= 1.096X ± 0.034
Intercept	= -2.63
N	= 100
MEAN (SYNCHRON LX Systems)	= 18.0
MEAN (Fluorescence Polarization Immunoassay)	= 18.8
CORRELATION COEFFICIENT (r)	= 0.983

As determined at UCDCMC

Serum (in the range of 5.0 to 27.8 µg/mL):

Y (DxC800-4449)	= 1.031X – 0.33
N	= 40
MEAN (DxC800-4449)	= 13.82
MEAN (Abbott Architect)	= 13.73
CORRELATION COEFFICIENT (r)	= 0.9956

Serum (in the range of 5.0 to 27.8 µg/mL):

Y (UniCel DxC800-4427)	= 1.035X – 0.75
N	= 40
MEAN (UniCel DxC800-4427)	= 13.46
MEAN (Abbott Architect)	= 13.73
CORRELATION COEFFICIENT (r)	= 0.9909

Serum (in the range of 5.0 to 27.8 µg/mL):

Y (UniCel DxC800-4118)	= 1.037X – 0.65
N	= 40
MEAN (UniCel DxC800-4118)	= 13.59
MEAN (Abbot Architect)	= 13.73
CORRELATION COEFFICIENT (r)	= 0.9946

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Serum (in the range of 5.0 to 27.8 µg/mL):

Y (UniCel Dx C800-4449)	= 0.97X + 0.54
N	= 40
MEAN (UniCel Dx C800-4449)	= 13.82
MEAN (UniCel Dx C800-4427)	= 13.46
CORRELATION COEFFICIENT (r)	= 0.9950

Serum (in the range of 5.0 to 27.8 µg/mL):

Y (UniCel Dx C800-4427)	= 0.998X – 0.11
N	= 40
MEAN (UniCel Dx C800-4427)	= 13.46
MEAN (UniCel Dx C800-4118)	= 13.59
CORRELATION COEFFICIENT (r)	= 0.9969

Serum (in the range of 5.0 to 27.8 µg/mL):

Y (UniCel Dx C800-4449)	= 0.990X + 0.36
N	= 40
MEAN (UniCel Dx C800-4449)	= 13.82
MEAN (UniCel Dx C800-4118)	= 13.59
CORRELATION COEFFICIENT (r)	= 0.9974

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

Precision

A properly operating SYNCHRON[®] System(s) and UniCel Dx C System(s) should exhibit imprecision values less than or equal to the following:

Precision Values as determined by Beckman

Type of Precision	Sample Type	1 SD	Changeover Value ^a	%CV
		µg/mL	µg/mL	
Within-run	Serum/Plasma	1.3	33	4.0
Total	Serum/Plasma	2.0	33	6.0

^aWhen 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 UC DMC

Type of Precision	Sample Type	n	Mean (µg/mL)	1 SD	%CV
DxC800-4118 Within-run	Beckman TDM 1	20	8.13	0.28	3.4
	Beckman TDM 3	20	40.41	1.49	3.7
DxC800-4427 Within-run	Beckman TDM 1	20	7.83	0.24	3.1
	Beckman TDM 3	20	39.78	1.14	2.9
DxC800-4449 Within-run	Beckman TDM 1	20	8.76	0.30	3.5
	Beckman TDM 3	20	39.34	0.95	2.4

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Type of Imprecision	Sample Type	n	Mean (µg/mL)	1 SD	%CV
DxC800-4427 Day to Day	MAS ChemTrak 1	40	7.97	0.29	3.7
	MAS ChemTrak 3	40	40.47	1.38	3.4
DxC800-4449 Day to Day	MAS ChemTrak 1	40	7.85	0.25	3.2
	MAS ChemTrak 3	40	41.13	1.71	4.2

Comparative performance data for a SYNCHRON LX[®] System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below. (16) Each laboratory should characterize their own instrument performance for comparison purposes.

Comparative performance as determined by Beckman

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	8.2	0.44	5.3
	Serum	Control 2	1	80	21.6	0.37	1.7
	Serum	Control 3	1	80	36.2	0.69	1.9
Total	Serum	Control 1	1	80	8.2	0.58	7.0
	Serum	Control 2	1	80	21.6	0.48	2.2
	Serum	Control 3	1	80	36.2	0.89	2.5

^aThe serum point estimate is based on the pooled 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 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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Prepared By	Date Adopted	Supersedes Procedure #
Kathy Dagan	November, 2015	New Test previously run at STC

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
11/02/2015	New	kdagan		