

Thermo Scientific QMS[®] Vancomycin Immunoassay on HITACHI[®] 917 System

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Abstract

Vancomycin hydrochloride is a tricyclic glycopeptide derived from *Amycolatopsis orientalis*.¹ It has come to be known as the last line of defence in treatment of methicillin-resistant *Staphylococcus aureus* infections. This glycopeptide inhibits the growth of the bacterium by intervening in the cell wall synthesis thereby killing the bacterium. The peak therapeutic range for vancomycin is between 20 to 40 µg/mL, the trough being at 5 to 10 µg/mL. Side effects of vancomycin are deafness (ototoxicity) and renal failure (nephrotoxicity) at levels above the therapeutic range. The need for monitoring this drug thus warrants attention. Thermo Fisher Scientific has developed a homogeneous turbidimetric immunoassay that utilizes the Quantitative Microsphere System (QMS) technology for analyzing vancomycin in human serum or plasma. A six-point calibration curve is generated where high rates are observed with the lowest vancomycin concentration, while lowest rates are observed with the highest vancomycin concentration. Using the Thermo Scientific QMS Vancomycin calibrators (0.0, 5.0, 10.0, 25.0, 50.0 and 100.0 µg/mL), the calibration curve generated is stable for approximately four weeks. The sensitivity of the immunoassay is 0.42 µg/mL (0.29 µmol/L) while the least observable dose is 2.0 µg/mL (1.38 µmol/L). The precision data was determined as described in NCCLS protocol EP5-A using a tri-level human serum based commercial control containing vancomycin at 7.56 µg/mL (Total CV 4.97%), 22.27 µg/mL (Total CV 2.94%) and 42.13 µg/mL (Total CV 2.90%). Linearity by dilution and spike recovery studies (range from 0 to 100 µg/mL) showed recovery of vancomycin within 10% of the expected starting values. A cross-reactivity of less than 5% was observed for the vancomycin metabolite CDP-I at 100 µg/mL. Ten endogenous substances which were tested, displayed less than 10% recovery from the expected starting values. Correlation studies were performed using NCCLS protocol EP9-A. One hundred sixty patient samples consisting of plasma and serum and ranging from 0.0 µg/mL (0.0 µmol/L) to 90.32 µg/mL (62.32 µmol/L) were tested. Results from the QMS Vancomycin Immunoassay on the HITACHI 917 platform were compared with the QMS Vancomycin Immunoassay on HITACHI 717 platform, resulting in a slope of 0.93, y-intercept of 1.047, and correlation coefficient (R²) of 0.992. These QMS Vancomycin reagents are stable uncapped and on-board up to 62 days.

Introduction

Thermo Fisher Scientific has developed a homogenous turbidimetric immunoassay that utilizes the QMS technology for analyzing vancomycin in human serum or plasma. Vancomycin (Figure 1a) is a tricyclic glycopeptide derived from *Amycolatopsis orientalis*.¹ It has come to be known as the last line of defense in treatment of methicillin-resistant *Staphylococcus aureus* infections.² This antibiotic inhibits the growth of the bacterium by intervening in the cell wall synthesis, thereby killing the bacterium. The therapeutic range for vancomycin is between 20 to 40 µg/mL, the trough being at 5 to 10 µg/mL.³ Side effects of vancomycin are deafness (ototoxicity) and renal failure (nephrotoxicity) at levels above therapeutic range. Extensive review articles have been published which fully examine vancomycin's effectiveness and pharmacokinetics.¹ Vancomycin is absorbed minimally from the gastrointestinal tract. In the first 24 hours after intravenous dosing, the usual route of administration, about 90% of the vancomycin is excreted unchanged by the kidneys. The average half-life in patients with normal renal function is about six hours. Vancomycin is approximately 55% bound to plasma proteins. Therapeutic serum levels vary depending on the microorganism involved and the patient's tolerance to the drug.^{4,5} Vancomycin serum or plasma concentrations are monitored to guide therapy, since individual patient differences require dose changes that are difficult to predict. Monitoring serum or plasma levels of vancomycin decreases the frequency of serious toxic effects.

Methods & Materials

The immunoassay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the vancomycin antibody reagent (Figure 1b). The vancomycin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-vancomycin antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of agglutination of the particles. When a sample containing vancomycin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with the highest rate of agglutination at the lowest vancomycin concentration and the lowest agglutination rate at the highest vancomycin concentration.

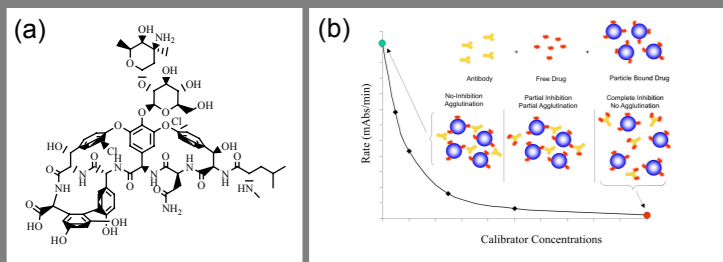
Reagents: R1 = Anti-Vancomycin Monoclonal Antibody (Mouse), R2 = Vancomycin-Coated Microparticles

Calibrators: QMS Vancomycin Calibrators A-F: 1x1mL each

Controls: BioRad Liquechek Immunoplus Controls

FIGURE 1. a) Structure of Vancomycin

b) Representation of Calibration Curve via the QMS Technology



Results

Specificity: Cross-reactivity and interfering substance studies were conducted using NCCLS protocol EP7-A as a guideline.⁶ Studies were conducted to examine the cross-reactivity of the QMS Vancomycin antiserum to the vancomycin metabolite Crystalline Degradation Product-I (CDP-I),⁷ commonly co-administered drugs, structurally related compounds and interfering substances. A 100 µg/mL concentration of CDP-I and structurally similar antibiotic, Teicoplanin, were tested (Table 1). Common and co-administered drugs (Table 2) and clinically high concentrations of endogenous substances (Table 3) were added to serum spiked with known levels of vancomycin and tested with the QMS Vancomycin Immunoassay along with serum controls. All compounds resulted in less than 10% error in detecting vancomycin.

TABLE 1. Cross-Reactivity with CDP-I & Teicoplanin

Substance	Concentration (µg/mL)	Percent Cross-Reactivity
CDP-I	100	4.64
Teicoplanin	100	0.99

TABLE 2. Cross-Reactivity with Common & Co-administered Drugs Tested at 500 µg/mL

Acetaminophen	Kanamycin A
Amikacin	Kanamycin B
Amphotericin B	Methotrexate
Ampicillin	Methylprednisolone
Bendroflumethiazide	Nalidixic Acid
Caffeine	Naproxen
Carbenicillin	Neomycin Sulfate
Cefamandole Nafate	Niacin
Cefazolin	Oxytetracycline
Cephalexin	Penicillin G
Cephalosporin C	Penicillin V
Cephalothin	Phenacetin
Chloramphenicol	Nitrofurantoin
Chlorothiazide	Prednisolone
Hemoglobin	Clindamycin
IgG	Erythromycin
HAMA Type-1	Ethacrynic Acid
HAMA Type-2	Ethambutol
Heparin	5-Fluorouracil
Rheumatoid Factor	Furosemide
Triglycerides	1100 IU/mL Sulfadiazine
	1000 mg/dL Sulfamethoxazole
	Gentamicin Sulfisoxazole
	Hydrochlorothiazide Tetracycline
	Ibuprofen Tobramycin
	Isoniazid

TABLE 3. Endogenous Substances Tested

Substance	Concentration
Albumin	10 g/dL
Bilirubin	70 mg/dL
Cholesterol	500 mg/dL
Hemoglobin	1150 mg/dL
IgG	6 g/dL
HAMA Type-1	-
HAMA Type-2	-
Heparin	500 units/mL
Rheumatoid Factor	1100 IU/mL
Triglycerides	1000 mg/dL

Precision: Precision was determined as described in NCCLS protocol EP5-A.⁸ A tri-level human serum based commercial control containing vancomycin at low, medium and high therapeutic values was used in the study. Each level of control was immunoassayed in duplicate twice a day for twenty days. Each of the runs per day was separated by at least two hours. Results are shown in Table 4.

TABLE 4. Precision Summary

	n	Mean	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Low Control	80	7.56	0.25	3.3%	0.26	3.4%	0.11	1.4%	0.38	5.0%
Mid Control	80	22.27	0.30	1.4%	0.37	1.7%	0.45	2.0%	0.66	2.9%
High Control	80	42.13	0.65	1.6%	0.79	1.9%	0.67	1.6%	1.22	2.9%

Linearity & Accuracy: Linearity (Figure 2, Table 5) and accuracy (Table 6) were determined using NCCLS guideline EP6-A.⁹ USP traceable vancomycin stock solution was diluted using human serum negative for the drug to achieve concentrations across the immunoassay range, such that 100.0, 75.0, 37.50, 7.50 and 2.50 µg/mL samples were obtained. Accuracy samples were prepared by diluting a stock concentration of vancomycin to several concentrations. Each sample was immunoassayed in triplicate using the QMS Vancomycin Immunoassay. The means of the replicates were determined for each sample and then plotted using the calculated target on the x-axis and the observed mean on the y-axis. Regression equations of the first and second order polynomial deviation were used to calculate the predicted first and second order values. A less than 10% difference was observed between the predicted first and second order values. QMS Vancomycin Immunoassay range is 2.0 µg/mL to 100.0 µg/mL.

FIGURE 2. Regression Analysis for Linearity Determination

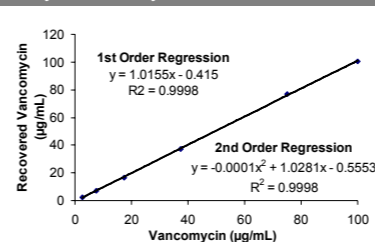


TABLE 5. Linearity

Calculated Target (x)	First and Second Order Comparison		% Deviation	
	Observed Mean (µg/mL)	Predicted First Order		Predicted Second Order
100.00	100.60	101.74	101.87	0.12%
75.00	76.69	77.46	77.70	0.32%
37.50	37.48	37.65	37.84	0.52%
17.50	16.80	16.64	16.69	0.26%
7.50	7.08	6.78	6.72	-0.83%
2.50	2.59	2.21	2.10	-4.93%

Acceptance Criteria: ±10% recovery between 1st and 2nd order predicted regression values.

TABLE 6. Accuracy

Calculated Target (x)	Observed				% Recovery
	Mean (µg/mL)	Std Deviation	% CV	% Recovery	
100.00	94.44	2.49	2.64	94.44%	
75.00	75.59	1.96	2.59	100.79%	
50.00	51.81	0.26	0.51	103.63%	
37.50	39.20	0.47	1.19	104.52%	
25.00	25.79	0.48	1.87	103.17%	
17.50	18.52	1.28	6.91	105.81%	
10.00	9.91	0.05	0.50	99.13%	
7.50	7.57	0.17	2.19	100.98%	

Acceptance Criteria: ±10% recovery

Sensitivity:

Least Detectable Dose (LDD): The LDD was determined to be 0.42 µg/mL.

The LDD, or analytical sensitivity, of the QMS Vancomycin Immunoassay is defined as the lowest drug concentration that can be distinguished from zero with 95% confidence. The LDD was determined by analyzing the lowest level calibrator, n = 20.

Limit of Quantitation (LOQ): The LOQ of the QMS Vancomycin Immunoassay is 2.0 µg/mL.

The LOQ, or functional sensitivity, of the QMS Vancomycin Immunoassay is defined as the lowest drug concentration for which acceptable intra-immunoassay precision is observed (often considered less than or equal to 20% CV) and recovery is between 85 to 115% of the theoretical value. The LOQ was determined by analyzing diluted samples twice (n=2) on 10 calibration curves over five days with the QMS Vancomycin Immunoassay. The study (Table 7) was conducted on more than one HITACHI 917.

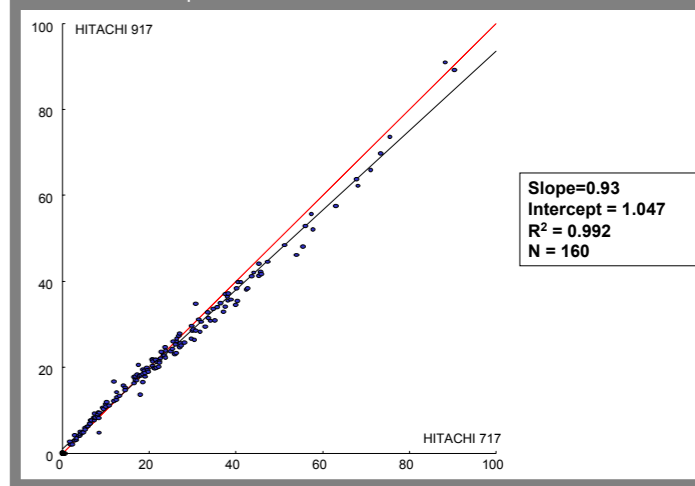
TABLE 7. Limit of Quantitation (LOQ)

Sample Concentration (µg/mL)	0.5	1	2	3	4	5
AVG (µg/mL)	0.27	0.86	2.19	3.35	4.17	5.49
n	20.00	20.00	20.00	20.00	20.00	20.00
SD	0.38	0.38	0.39	0.33	0.42	0.51
Percent CV	104.74%	44.19%	17.81%	9.85%	10.07%	9.29%
Percent Recovery	54%	86%	110%	112%	104%	110%

Method Comparison:

Correlation studies were performed using NCCLS protocol EP9-A as a guideline.⁹ Serum or plasma samples (N=160) from patients on vancomycin therapy were tested by the QMS Vancomycin Immunoassay on both the HITACHI 917 and HITACHI 717. The correlation coefficient (R²) for the comparison was 0.992 and the slope of the Passing-Bablok¹⁰ regression analysis was 0.93 and the y-intercept was 1.047.

FIGURE 3. Method Comparison of HITACHI 717 vs 917



Stability

*QMS Vancomycin reagents display up to twelve months of real time stability at 2 to 8°C.

*Reagent stability on a HITACHI platform is 62 days when uncapped and on-board.

*Calibration curve is stable for four weeks on HITACHI platforms.

Conclusions

*Performance results of the QMS Vancomycin Immunoassay on the HITACHI 917 show that the assay provides precise and reliable vancomycin concentrations within the therapeutic range to aid in optimal patient care.

*The QMS Vancomycin reagents and calibrators are liquid stable and are easy to use.

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