510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k063045

B. Purpose for Submission:
New Device

C. Measurand:
Vancomycin

D. Type of Test:
Homogeneous Enzyme Immunoassay

E. Applicant:
Diagnostic Products Corp.

F. Proprietary and Established Names:
IMMULITE ® 2000 Vancomycin,
IMMULITE ® 2500 Vancomycin

G. Regulatory Information:

1. Regulation section:
   21 CFR 862.3950
2. Classification:
   Class II, LEH
3. Panel:
   Toxicology (91)

H. Intended Use:

1. Intended use(s):
   Refer to Indications for Use below

2. Indication(s) for use:

   For *in vitro* diagnostic use with the IMMULITE 2000 Analyzer – for the quantitative measurement of vancomycin in serum and plasma (EDTA or heparinized), as an aid in monitoring the therapeutic administration of this antibiotic.

   For *in vitro* diagnostic use with the IMMULITE 2500 Analyzer – for the quantitative measurement of vancomycin in serum and plasma (EDTA or heparinized), as an aid in monitoring the therapeutic administration of this antibiotic.

3. Special conditions for use statement(s):
   For prescription use only.

4. Special instrument requirements:
   Immulite 2000 or Immulite 2500 analyzer

I. Device Description:

Immulite 2000/2500 Vancomycin Assay kit includes the following components:

1. Reagent wedge: With barcode. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to monoclonal murine anti-vancomycin.

2. Bead packs: With barcode. 200 beads, coated with ligand-labeled vancomycin.

3. Adjustors: Two vials (low and high) of lyophilized vancomycin in a human serum/buffer matrix.

4. Sample diluent: 50 mL concentrated (ready-to-use) vancomycin-free buffer matrix.

All human source materials were tested and found to be negative for syphilis, HIV 1/2, HBsAg, and HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   AxSYM® Vancomycin II
2. Predicate K number(s):
   k955851
3. Comparison with predicate:

A summary of the features of the IMMULITE 2000/IMMULITE 2500 Vancomycin assay and the predicate device is presented below.

Item	IMMULITE 2000/2500	AxSYM Vancomycin II
Intended Use	For *in vitro* diagnostic use with the IMMULITE 2000 or IMMULITE 2500 Analyzer – for the quantitative measurement of vancomycin in serum and plasma (EDTA or heparinized), as an aid in monitoring the therapeutic administration of this antibiotic.	The AxSYM Vancomycin II assay is a reagent system for the quantitative measurement of vancomycin, an antibiotic drug, in serum or plasma. The measurements obtained are used in the diagnosis and treatment of vancomycin overdose and in monitoring levels of vancomycin to ensure appropriate therapy.
Reportable Range	3.0 μg/mL – 50 μg/mL	3.0 μg/mL – 100 μg/mL

Item	IMMULITE 2000/2500	AxSYM Vancomycin II
Analytical Sensitivity (limit of blank, detection)	0.4 μg/mL Limit of Blank 0.9 μg/mL Limit of Detection	2.00 μg/mL (analytical sensitivity)
Sample Volume	10 μL IMM 2000 10 μL IMM 2500	Varies depending on the type of sample container. For sample cups, 150 μL (STAT: 94 μL). Minimum volumes calculated by AxSYM System.
Sample Type	Serum and plasma (heparin, EDTA)	Serum and plasma (sodium heparin, citrate, EDTA, oxalate)
Interferences	No significant interference from: Bilirubin up to 200 mg/L Hemoglobin up to 600 mg/dL Triglycerides up to 3000 mg/dL	Less than 10% interference from: Bilirubin up to 20 mg/dL Hemoglobin up to 1.0 g/dL Triglycerides up to 2300 mg/dL Total Protein from 3 - 10 g/dL
Calibration Adjustment Interval	2 weeks	Per AxSYM System Operator's Manual

K. Standard/Guidance Document Referenced (if applicable):

1) CLSI document EP5-A2 *Evaluation of Precision Performance of Quantitative Methods; Approved Guideline-Second Edition.*
2) CLSI document EP17-A *Protocols for the Determination of Limits of Detection and Limits of Quantitation*; Approved Guideline.
3) CSLI document EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline- Second edition.*

L. Test Principle:

The IMMULITE 2000, IMMULITE 2500 Vancomycin assay is a solid phase competitive chemiluminescent enzyme immunoassay. The solid phase (bead) is coated with ligand-labeled vancomycin. The reagent contains alkaline phosphatase (bovine calf intestine) conjugated to monoclonal murine anti-vancomycin. Vancomycin in the patient sample competes with the ligand-labeled solid phase for vancomycin binding sites on the monoclonal murine anti-vancomycin enzyme conjugate. The excess sample and reagent are removed by a centrifugal wash. Finally, chemiluminescent substrate is added to the bead and signal is generated in proportion to the bound enzyme.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The sponsor performed precision studies based on CLSI document EP5-A2. Two instruments were used on each platform (Immu 2000 and Immu 2500) with three different reagent kit lots on Immulite 2000 and one kit lot on Immulite 2500. Two aliquots of each test sample were assayed in two runs per day over 20 different days (not necessarily consecutive) for a total 80 replicates per test sample per lot. Two controls samples and six precision pools (human serum spiked with vancomycin with targeted concentrations of 2, 5, 10, 20, 30, 45 µg/mL) were used. The lowest pool sample consistently read lower than 3 µg/mL and was therefore not included in the analysis. The analysis of variance was used to estimate the within-run and total variance of the Immulite 2000 and 2500 Vancomycin assays. Summary of the results are shown below:

For the IMMULITE 2000, maximum statistics across 3 lots using 2 instruments per lot indicate that intra- and inter-assay CV% over the range of approximately 5 to 47 µg/mL are not greater than 10.2% and 6.8%, respectively.

For the IMMULITE 2500, maximum statistics for one kit lot using 2 instruments indicate that intra- and inter-assay CV% over the range of approximately 5 to 45 µg/mL are not greater than 6.1% and 6.0%, respectively.

The package insert claims for Immulite 2000 and 2500 Vancomycin assay precision are presented in the tables below:

Table 1: Immulite 2000 Precision (µg/mL)*

		Within-Run			Total	
Sample	N	Mean	SD	CV	SD	CV
Serum pool	80	5.12	0.32	6.3%	0.52	10.2%
Serum pool	80	10.2	0.69	6.8%	0.82	8.2%
Serum pool	80	20.4	1.10	5.4%	1.40	6.9%
Control	80	33.0	2.02	6.1%	2.54	7.7%
Serum pool	80	47.1	2.72	5.8%	3.18	6.8%

Table 2: Immulite 2500 Precision (µg/mL)*

		Within-Run			Total	
Sample	N	Mean	SD	CV	SD	CV
Serum pool	80	5.06	0.22	4.3%	0.31	6.1%
Serum pool	80	9.95	0.50	5.0%	0.56	5.6%
Serum pool	80	19.8	0.72	3.6%	0.92	4.6%
Control	80	32.2	1.37	4.3%	1.69	5.2%
Serum pool	80	45.0	2.37	5.3%	2.69	6.0%

* Representative of one kit lot on one instrument

b. *Linearity/assay reportable range*:

A study to evaluate the measuring range was performed on the Immulite 2000 using one kit lot with ten serum patient samples. A serial dilution was performed with each sample and all samples were run in triplicate. The range of the samples was 9 to 46 µg/mL. The mean result for each dilution was compared to its calculated expected value and expressed as a percent recovery. Recovery ranged from 93 to 105%. The average % recovery for these samples tested was 96%.

A recovery study was performed to test the ability of an assay to quantitatively recover added analyte. Six (6) patient samples and one calibrator were spiked with various concentrations of vancomycin on the Immulite 2000 analyzer. Recovery ranged from 93 to 110%. The average % recovery for these spiked samples was 101%.

In addition, sponsor also performed additional recovery study to confirm the manual dilution of 1:2 can be used when result was greater than 50 µg/mL(upper reportable range). Four samples with Vancomycin levels of 58.2, 91.9, 90.2, and 86.9 µg/mL were diluted and the mean % recovery was 90%. Dilution recovery ranged from 80 to 104%.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The Immulite 2000/ Immulite 2500 Vancomycin assay is traceable to the Chemical Reference Standard (CRS batch 2) certified by the European Pharmcopoeia Commision.

The Vancomycin adjustors, included in the reagent kit, are two levels (low and high) of lyophilized vancomycin in a human serum/buffer matrix. Adjustors are used to correlate the signal counts per second (CPS) of the IMMULITE platform instrument in the user's lab to those of the Master Curve and to account for the changes in reagent enzyme activity and/or operating conditions. In all IMMULITE platform instruments, calibrators are used at the site of manufacture to establish the Master Curve, which is encoded in the kit barcode label. The calibrators are not provided to the customers because the calibration of a specific kit lot is completed at the DPC manufacturing site. Adjustors are used to correlate the signal counts per second (CPS) of the particular IMMULITE instrument in the user's lab to those of the Master Curve and to account for the changes in reagent enzyme activity and/or operating conditions. The quality of the adjustment is monitored by reviewing the slope and the intercept of the adjustment process, not the target values of the adjustors. The acceptance criteria of the slope and the intercept are specified in the Acceptability Criteria section in the specific IMMULITE platform instrument Operator's Manual. Therefore, concentrations of the adjustors are not provided to customers.

Stability of the kit: Stability testing is on-going. Results of the real-time and accelerated stress studies support claim of 360 days shelf life for the Immulite 2000/2500 Vancomycin assay kits when stored at 2-8^{O}C. Reagents are stable at 2-8^{O}C until expiration date and has an on-board stability of 90 days. Adjustors are stable at 2-8^{O}C for 30 days after reconstitution, or for 6 months (Aliquotted) at -20^{O}C.

d. Detection limit:

The Limit of Blank:
The determination of Limit of Blank (LoB) was based on the CSLI guidelines EP17-A. The guideline defines LoB as the highest value expected to be seen in a series of results for samples that contain no analyte. 60 replicates of three zero analyte samples were analyzed. The samples used were a zero analyte plasma sample, a zero analyte serum sample, and the assay's zero calibrator. The procedure was performed for three kit lots on the Immulite 2000 and one kit lot on the Immulite 2500. The LoB was computed parametrically as the mean minus 1.65*SD. The mean from the multiple instruments, kit lots and samples was calculated. The sponsor claim the LoB = 0.4 μg/mL

The Limit of Detection:
The determination of Limit of Detection (LoD) was based on the CSLI guidelines EP17-A. The guideline defines the LoD as the actual concentration at which an observed test result is likely to exceed the LoB and may therefore be declared as detected. 5 samples were tested and analyzed. A series of results (64) for each of the 5 samples were generated in a total of 8 runs on 8 unique days. There were 2 replicates of each sample per run. Two Immulite 2000 and two Immulite 2500 instruments were used in this procedure. The LoD was estimated as LoB + 1.65*SD. The mean result from each instrument for each sample used, for each kit lot was calculated. The sponsor claim the LoD = 0.9 μg/mL

The reportable range for the assay is 3 – 50 μg/mL.

e. Analytical specificity:

Potential cross-reactants were spiked at concentrations listed in the following table into a neat normal human serum sample and a human serum sample spiked with 25 μg/mL vancomycin. Both neat and spiked aliquots of the samples were analyzed with 2 replicates per sample. The average results were analyzed for percent interference/cross-reactivity. This procedure was carried out on one Immulite 2000 instrument using on lot of reagent. Results are presented below for potential cross-reactants tested in the concentration listed:

Potential Cross Reactant	Concentration of potential cross-reactant (µg/mL)	Sample Tested[1]			Cross-Reactivity[2]
		# of Replicates	Obs. Mean Result After Spike	SD (CV%)	
Acetaminophen	500	2	23.47	0.30 (1.3)	ND
Amikacin	500	2	22.89	0.13 (0.6)	ND
Ampicillin	500	2	22.56	0.18 (0.8)	ND
Amphotericin B	500	2	23.85	0.36 (1.5)	ND
Bendroflumethiazide	500	2	22.99	0.31 (1.3)	ND
Caffeine	500	2	22.73	0.41 (1.8)	ND
Carbenicillin	500	2	22.12	1.03 (4.7)	ND
Cefamandole Nafate	500	2	23.91	0.72 (3.0)	ND
Cefazolin	500	2	23.01	0.35 (1.5)	ND
Cephalexin	500	2	23.35	0.20 (0.9)	ND
Cephalosporin C	500	2	22.69	1.27 (5.6)	ND
Cephalothin	500	2	23.49	0.71 (3.0)	ND
Chloramphenicol	500	2	23.13	0.80 (3.5)	ND
Chlorothiazide	500	2	22.28	0.64 (2.9)	ND
Ciprofloxacin	500	2	23.32	0.78 (3.3)	ND
Clindamycin	500	2	22.30	0.34 (1.5)	ND
Crystalline Degradation Product-1 (CDP-1)	10	2	24.36	1.10 (4.5)	ND
Crystalline Degradation Product-1 (CDP-1)	20	2	23.33	0.57 (2.4)	ND
Crystalline Degradation Product-1 (CDP-1)	25	2	24.01	1.03 (4.3)	ND
Crystalline Degradation Product-1 (CDP-1)	50	2	22.96	0.74 (3.2)	ND
Crystalline Degradation Product-1 (CDP-1)	100	2	23.05	0.52 (2.3)	ND
Erythromycin	500	2	23.41	0.82 (3.5)	ND
Ethacrynic Acid	500	2	22.89	0.76 (3.3)	ND
Ethambutol	500	2	23.31	0.83 (3.6)	ND
5-Fluorocytosine	500	2	23.15	0.88 (3.8)	ND
Furosemide	500	2	23.30	0.49 (2.1)	ND
Fusidic Acid	500	2	23.43	0.32 (1.4)	ND
Gentamycin	500	2	23.54	0.36 (1.5)	ND
Sodium heparin	500	2	23.53	0.25 (1.1)	ND
Hydrochlorothiazide	500	2	24.32	1.45 (6.0)	ND
Ibuprofen	500	2	24.17	0.59 (2.4)	ND
Isoniazid	500	2	23.97	0.26 (1.1)	ND
Kanamycin A	500	2	23.70	0.57 (2.4)	ND
Kanamycin B	500	2	22.54	0.33 (1.5)	ND
Lincomycin	500	2	22.15	0.91 (4.1)	ND
Methylprednisolone	500	2	23.60	0.31 (1.3)	ND
Methotrexate	500	2	22.06	0.00 (0.0)	ND
Nalidixic Acid	500	2	23.66	2.02 (8.5)	ND
Naproxen	500	2	22.10	0.25 (1.1)	ND
Neomycin	500	2	22.02	0.13 (0.6)	ND
Netilmicin	500	2	22.75	0.04 (0.2)	ND
Niacin (Nicotinic Acid)	500	2	22.29	0.15 (0.7)	ND
Nitrofurantoin	500	2	22.94	0.57 (2.5)	ND
Oxaprozin	500	2	23.49	0.04 (0.2)	ND
Oxytetracycline	500	2	23.20	0.49 (2.1)	ND
Penicillin G Potassium Salt	500	2	22.51	0.11 (0.5)	ND
Penicillin V Potassium Salt	500	2	22.56	0.65 (2.9)	ND

[1] 25 µg/mL vancomycin added to human serum sample

[2] ND = Not Detectable

Potential Cross Reactant	Concentration of potential cross-reactant (µg/mL)	Sample Tested[1]			Cross-Reactivity[2]
		# of Replicates	Obs. Mean Result After Spike	SD (CV%)	
Phenacetin	500	2	23.00	1.19 (5.2)	ND
Prednisolone	500	2	22.97	1.17 (5.1)	ND
Prednisone	500	2	22.32	0.06 (0.3)	ND
Rifampin	500	2	22.92	0.70 (3.1)	ND
Salicylic Acid	500	2	23.90	0.14 (0.6)	ND
Sisomicin	500	2	23.13	0.78 (3.4)	ND
Spectinomycin	500	2	23.52	0.79 (3.4)	ND
Streptomycin	500	2	23.47	0.28 (1.2)	ND
Sulfadiazine	500	2	22.34	0.54 (2.4)	ND
Sulfamethoxazole	500	2	24.11	1.69 (7.0)	ND
Sulfisoxazole	500	2	23.75	0.92 (3.9)	ND
Teicoplanin	10	2	24.80	1.10 (4.4)	ND
Teicoplanin	25	2	23.62	0.74 (3.1)	ND
Teicoplanin	50	2	24.65	1.08 (4.4)	ND
Teicoplanin	100	2	23.41	0.11 (0.5)	ND
Tetracycline	500	2	22.11	0.25 (1.1)	ND
Ticarcillin	500	2	23.93	0.01 (0.0)	ND
Tobramycin	500	2	23.65	0.39 (1.6)	ND
Trimethoprim	500	2	23.18	0.06 (0.3)	ND

The sponsor also analyzed the effects of bilirubin, hemoglobin, lipemia (using fat emulsion), albumin, cholesterol, IgG, Heparin, HAMA and rheumatoid factor on the assay. Neat and spiked aliquots of the samples were analyzed with 2 replicates per sample. The average results of spiked samples were inspected for differences from their neat counterparts. The sponsor claims there is no cross reactivity/interference from the concentrations tested below:

Bilirubin (icterus) up to 200 mg/L
Hemoglobin up to 600 mg/dL
Triglycerides (lipemia) up to 3000 mg/dL
Albumin up to 10g/dL
Cholesterol up to 500 mg/dL
IgG up to 6g/dL
Heparin up to 500 USP units/mL
HAMA up to 1880 ng/mL
Rheumatoid Factor up to 2330 IU/mL

The sponsor states in the limitation section of their labeling the following: "Heterophilic antibodies in human serum/plasma can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. ……For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings."

f. Assay cut-off:

Not Applicable.

2. Comparison studies:

a. Method comparison with predicate device:

162 human specimens obtained from the commercial suppliers were used in the method comparison study with the predicate device (Axsym Vancomycin II). All samples were assayed in singlicate for 3 different kits lots on the Immulite 2000 and one kit lot on the Immulite 2500 analyzer. Regression analyses using linear least squares correlation, Deming and Bland-Altman difference plots were provided. Results are summarized below:

1. Immulite 2000 Vancomycin (Y) vs Axsym Vancomycin II (X):

   Linear Least Squares regression: Y= 1.02 X + 0.73; r = 0.97, n= 162. Sample range from 3.7 to 38.6 µg/mL.

2. Immulite 2500 Vancomycin (Y) vs Axsym Vancomycin II (X):

   Linear Least Squares regression: Y= 1.03 X + 0.49; r = 0.97, n= 162. Sample range from 3.7 to 38.6 µg/mL.

3. Immulite 2500 Vancomycin (Y) vs Immulite 2000 Vancomycin (X):

   Linear Least Squares regression: Y= 0.98 X + 0.17; r = 0.97, n= 162. Sample range from 4.11 to 39.8 µg/mL.

b. Matrix comparison:

A comparison study was performed using 42 matched serum (plain and SST) and plasma (EDTA and heparin) samples. In all 42 matched set samples, equal volumes were spiked with various concentrations of vancomycin to obtain values from <3 µg/mL to 70 µg/mL. Only samples within the reportable range of 3-50 µg/mL were included in the regression analyses. Regression analyses using linear least squares correlation, Deming and Bland-Altman difference plots were provided. Results are summarized below:

1. Serum Separator tube (SST) (Y) vs Plain serum (X):

   Linear Least Squares regression: Y= 1.00 X - 0.07; r = 0.99, n= 33

2. Lithium Heparin plasma (Y) vs Plain serum (X):

   Linear Least Squares regression: Y= 1.02 X + 0.03; r = 0.99, n= 32

3. EDTA plasma (Y) vs Plain serum (X):

   Linear Least Squares regression: Y= 1.00 X + 0.001; r = 0.99, n= 31

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not Applicable

   b. *Clinical specificity:*
      Not Applicable.

   c. *Other clinical supportive data (when a. and b. are not applicable):*
      Not Applicable

4. Clinical cut-off:

   Not Applicable.

5. Expected values/Reference range:

   The sponsor recommends vancomycin trough values of 5 – 10 µg/mL and peak values of 25 – 40 µg/mL as reported in the literature.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.