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

A. 510(k) Number:

k071455

B. Purpose for Submission:

New assay

C. Measurand:

Cyclosporine

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Siemens Medical Solutions

F. Proprietary and Established Names:

Advia Centaur® Cyclosporine (CsA), and Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MKW	Cyclosporine Test System	862.1235	Toxicology
DLJ	Clinical Toxicology Calibrator	862.3200	Toxicology

H. Intended Use:

1. Intended use(s):

   The ADVIA Centaur® Cyclosporine (CsA) assay is an *in vitro* diagnostic immunoassay for the quantitative determination of cyclosporine in human whole blood using the ADVIA Centaur® Systems. The assay is intended for use as an aid in the management of cyclosporine therapy in kidney, heart and liver transplant patients.

The ADVIA Centaur® Cyclosporine Calibrator is for *in vitro* diagnostic use in the calibration of the Cyclosporine assay on the ADVIA Centaur® System.

2. Indication(s) for use:

   See intended use

3. Special conditions for use statement(s):

   For prescription use. See "Expected range" section, below.

4. Special instrument requirements:

   ADVIA Centaur®

I. Device Description:

The ADVIA Centaur CsA kit contains one ReadyPack containing three primary components: Solid Phase, Ancillary Well and Lite reagents.

The CsA solid phase reagent contains streptavidin-coated magnetic particles stored in phosphate buffered saline with bovine serum albumin, mouse gamma globulin, surfactant, and preservatives.

The CsA Lite Reagent consists of acridinium ester labeled CsA in phosphate buffer with bovine serum albumin, surfactant, and preservatives.

The CsA Ancillary-Well reagent contains biotinylated mouse monoclonal anti-CsA antibody in phosphate buffered saline with bovine serum albumin, mouse gamma globulin, surfactant, and preservatives.

The CsA Sample Pretreatment reagent consists of a hypotonic solution of detergents, glycerol, anti-foam and preservatives.

Two levels of calibrators are sold separately from the CsA kit. They contain CsA in serum-based material, with detergent, preservatives, glycerol, and antifoam. The calibrator contains human source material. While each donor unit was tested by FDA-approved methods and found non-reactive to hepatitis B surface antigen, antibody to hepatitis C, and antibody to HIV-1/2, all products using human source material should be handled as potentially infectious. Because no test method can offer complete assurance that infectious agents are absent, these products should be handled according to established good laboratory practices.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Abbott TDx®/FLx® Cyclosporine Monoclonal Whole Blood Assay

2. Predicate K number(s):

   P890025 (Cyclosporine assays were subsequently down-classified into Class II)

3. Comparison with predicate:

   The two devices are similar in terms of intended use and indications for use. Both are immunoassays using mouse monoclonal antibodies. Antibodies, reagents, specific technologies, and instrumentation differ. The Advia Centaur assay uses chemiluminescence; the predicate uses fluorescence polarization. The Advia Centaur assay uses a 2-point calibration; the predicate device uses a 6-point calibration.

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

Clinical and Laboratory Standards Institute (CLSI) document EP5-A2

L. Test Principle:

The ADVIA Centaur Cyclosporine assay is a competitive immunoassay using direct chemiluminescent technology. Cyclosporine in the patient sample competes with acridinium ester-labeled cyclosporine for a limited amount of biotin-labeled monoclonal mouse anti-cyclosporine antibody. Biotin-labeled anti-cyclosporine binds to streptavidin that is covalently coupled to paramagnetic particles in the Solid Phase. In the ADVIA Centaur Cyclosporine assay the sample is manually pretreated to lyse the cells and solubilize the cyclosporine. An inverse relationship exists between the amount of cyclosporine present in the patient sample and the relative light units detected by the system.

M. Performance Characteristics (if/when applicable):

Precision and method comparison were determined at 3 external clinical sites, as well as at the manufacturer's site.

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision evaluations included external study sites, (internal) manufacturer's site, and an additional evaluation near the upper limits of the assay. Evaluations are described below:

External site imprecision evaluation:
At each of 3 external sites, assay precision was evaluated using 2 lots of calibrators and 2 lots of reagents, over 5 days. Each day, 5 replicates were evaluated in a single run. Each of the 5 replicates was separately pre-treated/extracted. Spiked whole blood samples, patient pools, and whole blood control materials were all evaluated at estimated concentrations shown in the table below. Precision estimates were derived for within-run, between-day and within-site (the latter includes within-run and between-day). Statistical analyses were based on CLSI Document EP-5A. Results of a representative lot and site are shown below. Results from other sites were similar.

Sample	# Replicates per site	Mean concentration ng/mL	WR %CV	Total %CV
Control 1	25	89	6.8	6.9
Control 2	25	182	5.1	5.6
Control 3	25	350	6.5	6.5
Control 4	25	643	6.9	7.9
Control 5	25	1153	5.7	9.5
Spiked whole blood	24	117	5.9	6.4
Spiked whole blood	25	341	7.2	7.7
Spiked whole blood	25	525	6.0	7.2
Spiked whole blood	25	649	5.8	6.3
Spiked whole blood	25	901	7.0	7.7
Patient pool	25	171	6.1	6.1
Patient pool	25	286	6.8	6.8
Patient pool	25	475	6.4	6.7

The *between-site* CV for the samples shown in the table above ranged from 5.3% (for concentrations near 171 ng/mL) to 7.7% (for concentrations above 1000 ng/mL).

Internal precision evaluation:
A 20-day study was performed according to CLSI, document EP05-A2. Three replicates of calibrators and controls were assayed on each run. Data for the 20 days were analyzed using 2-point stored (day 0) calibration (n=120). Results for 1 representative lot are shown below.

Sample	Mean (ng/mL)	Within -run		Between-run		Between-day		Total	
		SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV
Control 1	84.7	3.2	3.8	1.3	1.5	3.3	3.9	4.8	5.7
Control 2	181.1	5.2	2.9	4.8	2.7	2.0	1.1	7.4	4.1
Control 3	372.0	10.4	2.8	17.6	4.7	0.0	0.0	20.4	5.5
Control 4	639.0	26.1	4.1	33.8	5.3	0.0	0.0	42.7	6.7
Control 5	1022.8	47.4	4.6	79.2	7.7	0.0	0.0	92.3	9.0
Low Calibr	88.6	3.7	4.2	2.5	2.8	3.3	3.7	5.5	6.2
High Calibr	1340.5	58.5	4.4	84.3	6.3	0.0	0.0	103.7	7.7

Precision near assay upper limit:

To address precision near the upper limit of the assay five whole blood samples were spiked with cyclosporine to concentrations near the upper limit of the assay. The precision analysis was performed over the course of 6 days. Ten total runs were performed with a maximum of 2 runs per day. Results are shown below:

			Within-run		**Between-run**		**Between-day**		**total**	
Sample	**N**	**Mean**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Spiked WB	40	1541.19	46.59	3.0	97.65	6.3	0.00	0.0	108.20	7.0
Spiked WB	40	1490.03	56.73	3.8	41.80	2.8	26.56	1.8	75.30	5.1
CTRL2	20	177.39	5.19	2.9	0.00	0.0	3.38	1.9	6.19	3.5
CTRL3	20	387.54	5.65	1.5	8.08	2.1	8.92	2.3	13.30	3.4
CTRL4	20	815.19	26.85	3.3	0.00	0.0	18.10	2.2	32.38	4.0
CTRL5	20	1660.96	73.95	4.5	92.13	5.5	0.00	0.0	118.14	7.1

b. Linearity/assay reportable range:

Spiking recovery:

Cyclosporine was spiked into two cyclosporine-free whole blood samples and 2 whole blood samples from patients treated with cyclosporine. Sample were then pretreated and evaluated on the Advia Centaur. Expected concentrations, determined gravimetrically, ranged from 100-1500 ng/mL. Averaged recoveries at (Advia Centaur measurement/ Expected concentration) ranged from 91% - 108% of the expected value, with no apparent concentration-dependent trends.

As part of the limit of quantitation evaluation, additional whole blood samples, spiked to concentrations near the lower limit of the assay, were evaluated for recovery. Averaged observed recovery (n=24) was 95% relative to expected concentrations determined gravimetrically.

Dilution recovery:
Human whole blood samples were diluted to achieve concentrations across the entire assay range. The recoveries ranged 90.7 to 108.5%, with no apparent concentration-dependent trends (and averaged recovery 98%).

To validate the dilution recommendations for high samples, samples spiked to levels higher than the claimed assay range were diluted with diluent, as recommended in the package insert. Recoveries ranged from 91-107%.

The assay range is 30-1500 ng/mL based on the limit of quantitation, and performance across this range in the analytical and method comparison studies.

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

A two-point calibration is used for this method. The calibrators (two levels) are sold separately from the assay kit. Calibrators do not need to be pretreated prior to analysis.

The ADVIA Centaur Cyclosporine assay is standardized to an internal standard manufactured using USP grade cyclosporine. Assigned values of calibrators and controls are traceable to this standardization. Percent differences observed between the control doses as read off multiple calibrator lots was within 5%.

Calibrator stability –Calibrator stability testing over the expiration dating period demonstrated that results were within 10% of the day 0 or same day frozen calibrator through 108 weeks.

d. Detection limit

A Limit of Quantitation study was modeled from CLSI EP-17with the time limitation of using whole blood samples. Samples of whole blood from 5 donors not taking cyclosporine were spiked to varying concentrations at low levels, down to 12.5 ng/mL cyclosporine. The whole blood samples were mixed and left to equilibrate overnight. Each spiked sample was extracted in replicates of 5 within each day and this procedure was run for 5 days. The evaluation used stored calibration from day 1. Performance estimated at 30 ng/mL includes within-lab precision of < 20% and averaged recovery of within 95%.

e. Analytical specificity:

Cross-reactivity:
Cyclosporine-negative whole blood samples were spiked with ~200 ng/mL of

cyclosporine and 1000 ng/mL of each of the following metabolites: AM1, AM1c, AM4n, AM9 and AM19. All 5 metabolites used in this experiment had purity >95% as assessed by HPLC. Testing was across 3 reagent lots. The percents cross-reactivity was calculated using the equation:

$$\%\text{Cross-reactivity} = (([\text{Metabolite}]_{\text{observed}} - [\text{Control}]_{\text{observed}})/ [\text{Metabolite}]_{\text{added}})) \times 100$$

The following cross-reactivity ranges were observed for the 3 lots:
AM1: 0.6-2.3%
AM1c: < 1%
AM9: 14-16%
AM19: ≤ 1.4%
AM4n: ≤ 1.7%

Potential interference by exogenous compounds:
Whole blood samples containing 200 ng/mL of cyclosporine were spiked with potentially co-administered drugs and other exogenous compounds to concentrations exceeding what would be expected in the intended use population. ADVIA Centaur Cyclosporine assay results from the spiked samples were compared with those of unspiked control samples. Observed recoveries were within 92-107%. The compounds tested and their concentrations are listed in the product package insert.

Endogenous compounds:
Whole blood EDTA samples were spiked with cyclosporine to a final concentration near 200 ng/mL and potentially interfering endogenous compounds were added. Some interference was observed at high concentrations of triglycerides and cholesterol:

Triglycerides:	0 mg/dL	control
	350 mg/dL	111% recovery
	500 mg/dL	92% recovery
	900 mg/dL	87%
Cholesterol	0 mg/dL	control
	250 mg/dL	97%
	300 mg/dL	90%
	400 mg/dL	87%

The manufacturer includes the following limitations in the package insert:

High levels of triglycerides and cholesterol may result in low quantitation in lipemic samples.

Average (n=3) percents recovery for cyclosporine in the presence of other endogenous compounds are shown below:

Bilirubin	103% recovery at 40 mg/dL
Urea	90% recovery at 20 mg/dL

Albumin	97% recovery at 8 mg/dL
Biotin	95% recovery at 50 mg/dL
IgG	96% recovery at 12 mg/dL

Varying hematocrit levels between 12% and 59% were evaluated. Recoveries ranged from 110% (for 12% hematocrit) to 98% (for 59% hematocrit).

The heterophilic antibodies tested were not observed to interfere; however, the manufacturer includes the following limitation: Human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Routine exposure to animals or animal serum products can cause interference and anomalous values. Diagnosis may require additional information.

f. Assay cut-off:

A cutoff is not applicable for this quantitative assay.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were conducted at three external sites comparing the ADVIA Centaur CsA assay against Liquid Chromatography Mass Spectrometry (LCMS), as well as against the Abbott TDx®/TDxFLx® Cyclosporine Monoclonal Whole Blood immunoassay. Patient samples were selected to have known characteristics (age, gender, location, time post-transplant, time of blood-draw [C0, C2], transplant group). In addition, only EDTA whole blood stored < 2 days at 2-8 degrees C, or stored at -20 degrees C were selected. Samples were largely from adults in the age range of 32-70 (but also included a few samples outside this range). Samples were included from both men (n=165) and women (n=83), and were obtained from more than 3 locations. Samples represented both chronic (n=197) and acute (n=51) conditions as well as varying draw times (n=59, for C2 (peak); n=189 for C0 (trough)). Regression results for each of these subgroups are shown in the tables below.

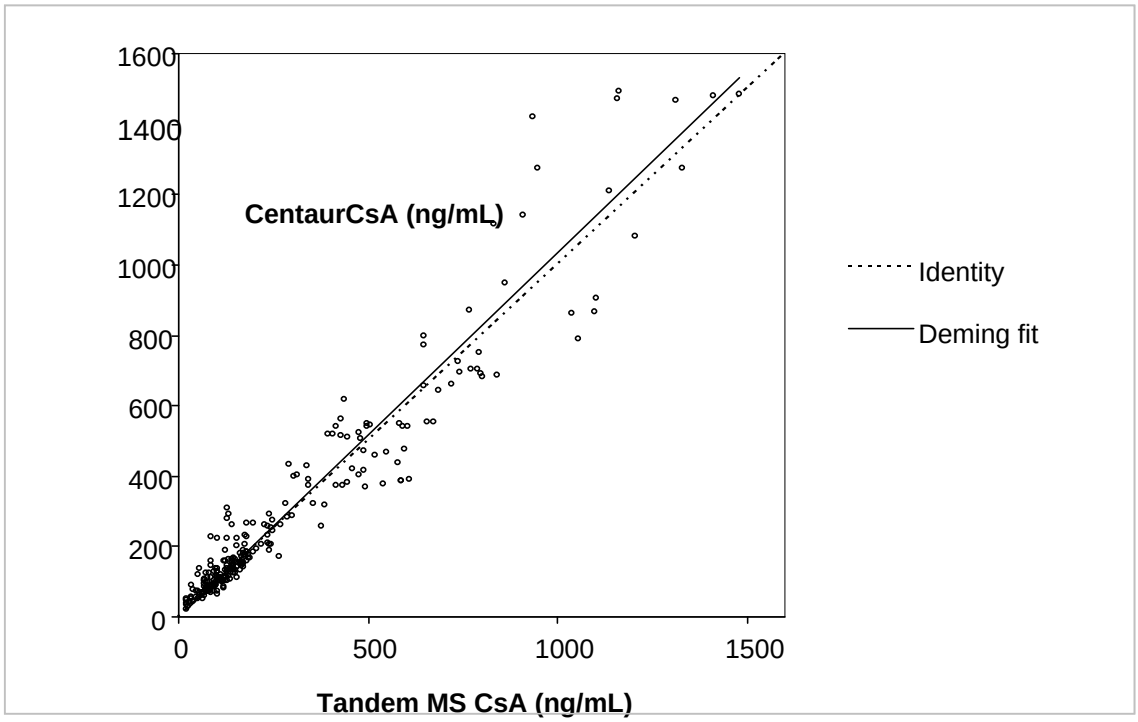
A summary of results, as determined by Deming Regression, are described in the following tables. Results are based on single measurements per sample.

Comparator	Transplant Type	# of Samples	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
Tandem MS	Kidney	108	1.1 (1.0 to 1.2)	-8 (-29 to 13)	0.96
Tandem MS	Liver	75	1.0 (0.9 to 1.2)	-5 (-31 to 21)	0.97
Tandem MS	Heart	67	0.9 (0.8 to 1.0)	20 (0 to 40)	0.97

Tandem MS	all	250	1.0 (1.0 to 1.1)	-1 (-16 to14)	0.96
Comparator	Site	# of Samples	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
Tandem MS	Site 1	97	0.9 (0.8 to 1.0)	-8 (0 to 27)	0.96
Tandem MS	Site 2	105	1.1 (1.0 to 1.2)	-5 (-32 to 2)	0.98
Tandem MS	Site 3	48	1.1 (0.9 to 1.4)	20 (-8 to 79)	0.96
Tandem MS	All	250	1.0 (1.0 to 1.1)	-1 (0.96 to 1.1)	0.96

Comparator	Peak/ Trough	# of Samples	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
Tandem MS	Trough	182	1.0 (0.8 to 1.1)	8 (0 to28)	0.96
Tandem MS	Peak	68	1.2 (1.0 to 1.3))	-104 (-208 to 0)	0.97
Tandem MS	All	250	1.0 (1.0 to 1.1)	-1 (1.0 to 1.1)	0.97

The graph below shows all data points (single measurements) combined for Centaur CsA versus Tandem-MS.



b. Matrix comparison:

Not applicable; the assay is for use with EDTA whole blood samples only.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable; clinical sensitivity and specificity are not typically reviewed for this device type.

b. Clinical specificity:

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

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

No firm therapeutic range exists for cyclosporine in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, co-administration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors will cause different requirements for optimal blood levels of cyclosporine. Each clinician should establish a range based on clinical experience, and evaluate each patient before treatment adjustments are made. In addition, ranges will vary according to the commercial *in vitro* diagnostic test used. Do not use conversion factors between commercial assays to predict values for individual patients. Consistent use of one assay for an individual patient is recommended because of varying patterns of cross-reactivity with metabolites.

Measurements of cyclosporine should be used in conjunction with other diagnostic procedures and clinical evaluation. Do not base changes in the cyclosporine treatment regimen on individual cyclosporine values.

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.